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(*PARALITHODES CAMTSCHATICA* TIL.)

YOSHIO NAGASAWA

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INTRODUCTION

Crab fishing is well known as the most important of Japan's fishing industries. Before World War II, the production of canned crab had reached 500,000 cases per year[1]. However, the canning industry in Japan stood on the brink of ruin during the war and the production was reduced to 12% of that before the war. After World War II, the fishing and canning industry again became prosperous with 420,000 cases being produced just as before the war.

Technological problems which arise during the manufacturing of canned crab and the studies on these problems are summarized as follows:

(1) Blackening of canned crab meat[2,3,4]
(2) Flat sour of canned crab meat[5]
(3) Swelling of canned crab[6]
(4) Blue meat in canned crab meat[7-10]
(5) Formation of struvite[11]
(6) Browning of canned crab meat

Among those problems, the phenomena as noted in items (1)~(5) have occurred frequently since early times in the manufacturing of canned crab.

The problems noted above with the exception of the "browning of canned crab meat" have been solved nowadays because of the latest improvements in technique. For example, in the preventing of the occurrence of blue meat[10], the "low temperature boiling method" was invented, and in the preventing of the formation of struvite[11] study shows rapid cooling or the addition of chemical reagents to be effective.

The "browning" of canned crab which has come to be recognized frequently in the crab canning industry since the War remains as an unsolved problem. The merchandise value of the can is remarkably reduced by the occurrence of "browning" and the tendency to suffer from this phenomenon for trade in canned crab is becoming more and more pronounced[12]. It is considered especially serious that the occurrence of "browning" is not recognized only in the can prepared from unfresh raw meat but is observed also in the canned meat prepared from fresh raw meat which is boiled by the "low temperature boiling method" for preparation of boneless crab meat.

For the purpose of obtaining some knowledges on the "browning" of canned crab meat, some reports of these studies have already been published[13-17].

It has been ascertained[13] that the Maillard reaction was a cause of the "browning" of canned crab meat. The author will continue to study from the point of view of food technology about the "browning" of canned crab meat.

Before going further the author wishes to express his profound thanks to Prof. Eiichi Tanikawa and Assist Prof. Minoru Akiba for their helpful sugges-

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I. HISTORY

Among characteristic changes which occur during the processing and handling of certain foods, browning phenomena are observed frequently. The occurrence of the phenomena in various kinds of foods has been widely investigated[18,19] and the causal significance has been classified as shown in Table 1.

Table 1. Classification of the causal significance of the "browning" reaction in various foods

No.	Types of the "browning" reaction	Causal substances	Example of "browning"
I	Amino-carbonyl reaction (Maillard reaction)	Carbonyl compounds Amino compounds	Milk, Dried egg, Dried fruits, Juice of fruits, White Fish meat
II	Caramelization	Carbonyl compounds	Caramel
III	Autolytic oxidation	Polyphenols, Di- or poly-carbonyl compound, Unsaturated fatty acids	Vegetable, Fruits, Fat, Oil (fish oil)
	Enzymatic oxidation	Polyphenol, Chlorogenic acid, Catechin, Tannin, Leucoanthocyan	Fruits, Tea, Cocoa

As seen in Table 1, three types of "browning" reaction are recognized during the processing and handling of foods. Amino-carbonyl reaction which include the combinations of aldehydes, ketons or reducing sugars with amino acids, peptides, or protein is known as the most common cause of "browning" of some foods.

Another type of "browning", called caramelization, occurs when polyhydroxy-carbonyl compounds (sugars, polyhydroxycarbonylic acids) are heated to relatively high temperatures in the absence of amino compounds. This type of "browning" reaction requires characteristically more energy to get started than the former type. At this point, caramelization is distinguished from amino-carbonyl reaction, but other properties are the same.

A third broad type of "browning" recognized frequently in oily or vegetable food is that caused by the group of oxidative reaction, ascorbic acid and polyphenols are converted into di- or polycarbonyl compounds. These oxidations may or may not be promoted by enzymatic action[20].

In starting these studies, the author assumed that the "browning" of canned

crab meat may rarely be caused by the third type described above, because the ungreasy boiled meat[21] in which a great amount of sugars or amino nitrogen is contained is packed into the empty can which is sealed under vacuum for sterilizing at relatively high temperatures (5.5~6 pounds pressure per square inch).

The "browning" phenomena which is caused by the combination between reducing sugars and amino compounds (especially amino acids) has since 1912 been well known as amino-carbonyl reaction or Maillard reaction[22].

The studies on the "browning" of agricultural products which is caused by the amino-carbonyl reaction have been made by many investigators[23-26].

However, there are a few papers on the "browning" of fish products[27,28]. According to Tarr, it was clarified that the "browning" occurred as a result of the combination between amino acids and ribose or glucose[28,29] and that the advance of the "browning" was influenced by the existence of some metals or by the hydrogen-ion concentration[30]. In this case, ribose seemed to be separated from ribonucleic acid, ribosied adenosine triphosphate and ribose-6-phosphate in fish meat[31].

The present author has studied the cause and mechanisms of the "browning" of the crab meat and has already reported the following items.

I. Difference in the chemical composition of normal and browned canned crab meat[13]

II. Effect of chemical materials on the "browning" reaction[14]

III. The "browning" of canned crab as dependent upon the time of leaving the raw crab without carapace but uncanned[15]

IV. Soaking of the raw material in water and heating[16]

V. "Browning" in different body-parts of raw crab[17]

In consequence of the studies stated above, it was made clear that the "browning" of the canned crab may be caused by the Maillard reaction by which amino acids and sugars in the crab meat are brought into combination with one another.

For the purpose of dissolving the "browning" of canned crab meat which is one problem in the crab canning industry today, it has been considered that further studies must be carried out in detail on the following items:

(1) Biochemical research on the quantitative change of the chemical components in the crab meat by which the "browning" is caused, when the crab is left standing as a raw material for the preparation of the canned crab.

(2) Detection of the chemical compounds which cause the "browning" of the canned crab.

(3) Isolation of the browned substance from the browned canned crab meat.

(4) The relation between the "browning" and each operation during the processing of canned crab.

In this paper the author will report his further studies concerning those problems where satisfactory results have been obtained.

II. EFFECT OF METALS ON THE "BROWNING" REACTION

In the previous paper[14], the effect of chemical materials such as sugars, amino acids and other organic compounds on the "browning" of canned crab meat was discussed. The validity of the suggestion that the "browning" of the meat may be caused by Maillard reaction was strengthened.

Tarr[29] has pointed out that the "browning" of cod meat caused by the Maillard reaction is affected by the presence of a few metals such as Cu and Fe.

In the study reported in this paper, the effect of metals on the "browning" of the meat was examined with samples prepared by adding various kinds of metals to the white leg meat of crab (*Paralithodes camtschatica*).

A. Effect of various metals on the "browning" reaction

1. *Preparation of samples used*

The crust of the leg meat of raw crab was removed with a knife. The meat was left in an ice box at a temperature of 3°C for 2 hours in order to let the blood flow out from the meat. After washing the material with a small quantity of water, the leg meat was removed from its red skin with forceps and knife and then used under the name of "white raw meat". The raw meat was homogenized and N/10 NaOH solution was added for adjusting pH to a 6.8 value. The amounts of free reducing sugar and amino acid in the meat were 32.5 *mg%* and 236.1 *mg%*, respectively.

2. *Determination of the "browning"*

Twenty *g* of the samples each mentioned above were put in Erlenmeyer flasks. To each flask, various kinds of metal salts as listed in Table 2 were added to a concentration of 1/100 Mol equivalent to the weight of the meat and each was mixed thoroughly. After being plugged with cotton, the flasks were heated at 110°C (6 pounds pressure) for 80 minutes.

After cooling, the degree of the "browning" of the white meat was estimated by Shimadzu's A.K.A. photoelectric colorimeter and the value of transparency was estimated.

3. *Results*

Whether "browning" of crab meat is affected by metals or not was examined. The results obtained are shown in Table 2.

As seen in Table 2, when cuprous, cupric, ferrous, and ferric chlorides were added to the white raw meat, the meat showed brown in color, but other chlorides of metals had absolutely no effect on the "browning" of the meat. The degree of "browning" was observed to be different depending on different valency of the metal ions which were added. The addition of cupric or ferrous chlorides,

as compared with the addition of cuprous or ferric chlorides, had a more remarkable influence upon the "browning" of the meat.

Table 2. Effect of the addition of various kinds of metallic salts on the "browning" of crab meat

Reagents added	Degree of browning of white crab meat (reflection ratio, %)	pH value of crab meat after heating
Blank	56.1	6.6
CuCl	22.0	6.2
$CuCl_2$	20.0	6.6
$FeCl_2$	27.0	6.8
$FeCl_3$	34.9	6.2
$CuCl_2$ and $FeCl_2$	19.1	6.4
KCl	53.0	6.6
AgCl	49.4	6.6
$SnCl_2$	55.0	6.2
$SnCl_3$	56.5	6.0
$ZnCl_2$	53.0	6.4
$CaCl_2$	52.0	6.6
$K_3Cu(CN)_4$	52.0	—
$K_3Fe(CN)_6$	42.0	7.0
KCN	43.0	7.0

It was of special interest that $K_3Cu(CN)_4$ containing Cu in its molecule had absolutely no influence on the "browning" of the meat. The occurrence of this interesting phenomenon is considered to be caused by the fact that $K_3Cu(CN)_4$ makes a complex ion of $Cu(CN)_4$ and does not dissociate Cu ion. Therefore, it is assumed that the addition of metal ions (such as Cu or Fe ions) influence the "browning" of the meat.

B. Effect of concentration of added metals on the "browning" reaction

1. *Preparation of sample*

By dissolving a definite amount of $CuSO_4$ or $FeCl_2$ in distilled water, cuprous sulphate or ferrous chloride solutions were separately prepared to contain 1 *mg* of Cu or Fe in 1 *cc* each of the solutions.

Various amounts of each solution were added separately to the "white raw crab meat" in order to prepare crab meat samples in which various amounts of Cu or Fe as described in Fig. 1 would be contained.

2. *Determination of the "browning"*

Ten *g* each of the crab meat prepared as above were put into Erlenmeyer flasks. After being plugged with cotton, the flasks were heated at 110°C (6 pounds pressure) for 80 minutes. After cooling, the degree of the "browning" of the meat was estimated as described above.

3. *Result*

The degree of the "browning" was measured in the meat containing various concentrations of Cu or Fe. The results obtained are shown in Fig. 1.

As observed in Fig. 1, the higher the amounts of Cu or Fe contained in the meat became, the more remarkable was the "browning" of the meat. The degree of "browning" was influenced remarkably more with the addition of Cu than with that of Fe. If in 100 *g* of the crab meat, more than 2.5 *mg* Cu or 5.0 *mg* Fe were added, the "browning" of the meat was easily recognized organoleptically. The meat which contained Cu of 5~10 *mg%* and above showed deep brown in color.

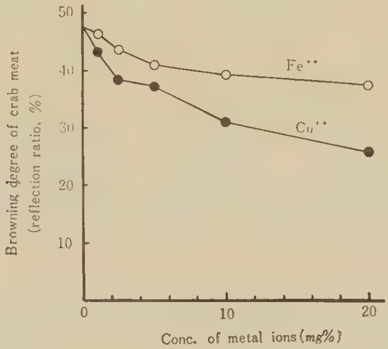


Fig. 1. Influence of the addition of Cu and Fe of various concentrations on the "browning" of crab meat

Therefore, action of the metals in causing the "browning" of canned crab is assumed to taken part in the process of the Maillard reaction which is suggested as a cause of the "browning" of the canned crab[13]. Such suspicion will be verified in a latter part of these studies.

III. VARIATION OF THE AMOUNTS OF VARIOUS CHEMICAL COMPONENTS BY TIME OF LEAVING THE CRAB CARCASS WITHOUT CARAPACE

In the previous paper[13-16], the author has asserted that there is an interesting relation between the "browning" of canned crab meat and the presence of free-amino nitrogen or free reducing sugar, such as diamino acid, glucose and ribose which were of special importance. Also, when the canned crab was prepared from unfresh raw material, it was clearly found that the qualities of the contents of the canned crab inevitably fall, and that "browning" takes place easily. The degree of the "browning" was not remarkably influenced according to the separate parts of the raw crab bodies[17].

In this experiment, the author tried to find the variation of various chemical components in each part of a crab body as dependent on the time of leaving the raw crab carcass without carapace.

1. *Sample used*

After being landed, the meat was classified into the separate parts such as leg meat, shoulder meat and claw meat. After the frozen crab meat had been defrosted, each part was left in an ice box at a temperature of 5°~8°C. Portions of the meat were employed at definite intervals to estimate the items below. As another sample, protein-free juice was prepared from leg meat by the same method as described in the previous paper[14].

By the addition of 30% tungstic acid solution to the juice, the juice was fractionated in Van Slyke's method into two fractions of monoamino and diamino acid parts[32]. They were heated at a pressure of 6 pounds for 90 minutes in order to estimate the degree of "browning".

2. *Items of analysis and method*

As in the previous paper[13], the analysis of the samples was carried out concerning the following items: (1) pH, (2) volatile basic nitrogen (V.B.-N), (3) amino nitrogen (NH_2-N), (4) reducing sugar, (5) kinds of free amino acids, (6) kinds of free sugars.

Table 3. Progressive variation of the amounts of chemical components

Items / Kinds of meat / Time of leaving the meat	pH			V.B.-N (*mg*%)		
	Leg meat	Shoulder meat	Claw meat	Leg meat	Shoulder meat	Claw meat
0 (hr.)	6.28	6.30	6.12	12.8	15.2	11.0
24	5.90	6.20	6.00	16.5	17.1	14.9
48	6.30	6.40	6.20	19.3	22.6	17.6
96	6.80	7.00	6.40	120.0	122.4	66.3

Table 4. Variation of the amount of amino nitrogen

Items / Kinds of meat / Time of leaving the meat	Amino nitrogen in forms of					
	Monoamino fraction (*mg*%)			Diamino fraction (*mg*%)		
	Leg meat	Shoulder meat	Claw meat	Leg meat	Shoulder meat	Claw meat
0 (hr.)	28.86	34.36	26.80	4.81	7.56	8.25
48	34.36	37.12	26.11	8.25	12.03	8.25
96	43.99	46.05	34.36	12.37	23.35	12.7

Detection of glucose or ribose was carried out by chromatographical method as in Tarr's studies[30,31]. The degree of the "browning" of two solutions mentioned above was also estimated with Shimadzu's A.K.A. photoelectric colorimeter for the value of transparency.

3. *Results*

Variation of the amount of chemical components was estimated as to the time of leaving the raw crab carcass without carapace; the results obtained are shown in Tables 3~5. The degree of "browning" was compared with two solutions in which monoamino and diamino acids were separately contained in the same concentration, and the results obtained are shown in Table 3.

As seen in Table 3, when each part of crab meat removed from carapace was left for a long period of time, pH of the meats decreased once in the initial time of being left (within 24 hours), but it increased gradually with the passage of time of being left beyond 24 hours. Volatile basic nitrogen, amino nitrogen, and reducing sugars gradually increased. However, the variation of the chemical components noted above seemed to be different with each part of the meat. Variation of pH and the amount of volatile basic nitrogen in the claw meat seemed to be smaller than that in other parts of the meat.

The amounts of amino nitrogen in the claw meat were smaller than those

in crab raw meat which is left for various intervals

NH_2–N (*mg%*)			Reducing sugar (mg%)		
Leg meat	Shoulder meat	Claw meat	Leg meat	Shoulder meat	Claw meat
185.8	153.7	112.6	10.0	13.0	—
193.7	167.1	138.4	19.6	30.8	20.0
198.6	171.7	148.6	37.5	37.0	41.0
222.9	175.8	158.3	42.0	50.0	52.0

by time of the crab carcass being left

$\frac{NH_2\text{–N in diamino frac.}}{NH_2\text{–N in monoamino frac.}} \times 100$ (%)			Identified free amino acids		
Leg meat	Shoulder meat	Claw meat	Leg meat	Shoulder meat	Claw meat
16.5	21.5	31.3	Gly. Pro.	Gly. His.	Gly. Pro.
24.0	32.5	31.6	{Arg. His. Gly. Glu. Pro.	Arg. His. Gly. Glu. Pro. Leu.	Pro. Gly. Glu. Pro. Ser.
28.1	50.7	37.0	{Arg. His. Ser. Gly. Glu. Thr. Pro. Tyr.	Arg. His. Ser. Gly. Glu.	

of the other parts of the crab meat. From these results, it may be suggested that claw meat is less subject to decomposition than other parts of the crab body.

As seen in Table 4, when the crab meat was left for a long period of time, the amount of amino nitrogen in monoamino and diamino fractions increased respectively, and the ratio of diamino nitrogen to monoamino nitrogen simultaneously showed tendency to increase. It was especially ascertained that the tendency of increasing in ratio was largest in the shoulder meat. There was no remarkable difference in the composition of free amino acids in each of the different parts of crab meat. However, progressive variations in free amino acids in each part of the crab meat apparently differed with the lengthening of each time period. In the initial period, neutral amino acids, such as glycine or proline were comprised mainly in free amino acids, and after the crab meat had been left more than 48 hours, basic amino acids, such as arginine or histidine appeared in free amino acids.

As seen in Table 5, the amounts of glucose or ribose in each part of the meat gradually increased, with the lengthening of the time. The amounts of ribose in claw meat seemed to be smaller than those in other parts, but there was no remarkably difference in the amounts of glucose in each part of the meat.

Table 5. Variation of the amounts of glucose and ribose by time of the crab carcass being left

Items / Kinds of meat / Time of leaving the meat	Amount of glucose in raw crab material			Amount of ribose in raw crab material		
	Leg meat	Shoulder meat	Claw meat	Leg meat	Shoulder meat	Claw meat
0 (hr.)	4.2	4.0	4.0	3.2	—	1.8
48	12.0	10.0	11.0	11.0	10.0	8.0
96	11.5	12.0	13.0	12.0	13.0	11.0

Table 6. Relation between the degree of the "browning" and kinds of amino acids isolated from crab meat

Sample / Items	Protein-free juice prepared from the leg meat			
	Monoamino fraction		Diamino fraction	
	before heating	after heating	before heating	after heating
Degree of browning (% of transparency)	88.0	14.0	88.0	7.0
Amount of reducing sugar (*mg%*)	180.0	—	180.0	—
Amount of amino nitrogen (*mg%*)	211.3	96.9	211.3	81.9

As seen in Table 6, in the "browning" reaction which occurred with the adding of glucose to monoamino acid or diamino acid solution, it was ascer-

tained that remarkable browning took place in the diamino acid solution.

When the raw crab meat without carapace (carcasses of crab) is left and the freshness of the meat falls as far as decomposition, the amounts of sugar and amino nitrogen in the meat are increased, especially of diamino acid, glucose, and ribose, which are factors causing the "browning". Therefore, it is easy to consider that the "browning" reaction in the canned crab prepared from unfresh raw meat in which a great amount of sugars and nitrogen substances are contained, easily progressed toward completion[33,34]

It is said in crab-canning factories that if the canned crab is processed from the raw material left for a long period, the "browning" is considered to occur easily in the canned contents of shoulder meat, and less likely to occur in the part of claw meat. However, this consideration is not in agreement with the results reported in the previous paper[17]. In the present author's opinion, it may be said that beyond the limit of freshness the claw meat will be brown as well as other parts (shoulder or legs).

Discrepancy in the mode of the change of amino nitrogen and sugars in the crab meat (carcass) left for a difinite period is certainly observed in the differences between monoamino fraction or diamino fraction, in that they are largest in the shoulder meat and smallest in the claw meat. Also, progressive variation of pH and volatile basic nitrogen in the claw meat is smaller than that in the other parts of the meat. From these observations, it may be suggested that claw meat is less subject to decomposition than other parts of the meat. These facts may result because claw meat is protected from direct bacterial attack and autolysis by enzyme and different histological construction from other parts.

IV. THE RELATION BETWEEN THE DIFFERENCE OF CHEMICAL COMPONENTS OF RAW CRAB MEAT WHICH EXIST BEFORE OR AFTER PEELING AND "BROWNING" OF CANNED CRAB

Peeling is a characteristic phenomenon in the life history of Crustacea. According to Marukawa[35], the peeling occurs all over the chitin parts of the body; by it the tendon in the leg meat is also renewed. As a result of peeling the length of the crab body increases about 40% in maximum.

The crust of the crab body immediately after the peeling is very soft, and the crab can not walk. But after a few days, the crust becomes pretty hard and the creature is able to walk again. After about 10 days, the body recovers strength[36].

The change which occurs before and after the peeling is seen not only in morphological appearance, but also in chemical components of the meat. For example, according to Shimoda[37], after the peeling changes of the amounts of crude fat and ash in the meat were remarkable; the amount of the ash was

minimum at the time of peeling.

The present author and Tanikawa[38] have compared the chemical components in the peeled-crab and the hard-shell crab meats. According to the results obtained, the amounts of water-content and crude fat in the peeled crab meat were larger than those in the hard-shell crab meat, but the amounts of crude protein and ash were the opposite. The amount of extractive nitrogen in the peeled-crab meat is considered to be less than that in the hard-shell crab meat. Therefore the taste of the former is inferior to that of the latter. It is often observed that the canned crab meat which is prepared from the peeled-crab generates few cases of "browning".

The chemical components of the meats of the peeled-crab have been compared with those of the hard-shell crab; also study was made of the difference of the components which are concerned with "browning" of the canned product.

1. *Preparation of sample meat*

The carapaces of hard-shell crab (carapace width 18.5 *cm*, weight 3.4 *kg*) and peeled-crab (width 16.0 *cm*, weight 2.1 *kg*) were removed from the bodies. Meat of each half carcass of the crabs which was removed from the crust was crushed in a homogenizer. Those samples of crushed meat were used for the estimation of the chemical components; the other half part of the carcasses of the crab was processed into canned crab by the following procedures.

2. *The processing of the sample cans*

Each half of the carcasses was divided into two parts; one part was processed by "high temperature boiling method", and the other by "low temperature boiling method", in separate quarter-pound cans.

In the "high temperature boiling method", the leg meat in the crust was boiled at 100°C for 18 minutes and then cooled. After the removing of the crust, the meat was processed by the usual method at a processing temperature of 109°C (5.5 pounds pressure) for 80 minutes. In the "low temperature boiling method", the leg meat in the crust was heated at 60°C for 10 minutes; then the meat was removed from the crust by cutting one side of the crust after cooling. The half-boiled meat was completely boiled at 100°C for 3 minutes, and the meat was processed in quarter-pound cans after cooling. The processing temperature was 109°C (5.5 pounds pressure) for 80 minutes. After the processing, the sample cans prepared by the two procedures were stored for about one month in the laboratory room.

After opening of the sample cans, the content was used for the experiments.

3. *Items estimated and the estimation method*

In order to know the difference of the chemical components of crab meat as they existed before and after the peeling, analysis was carried out concerning the following items.

(1) Water-content (by drying method), (2) Ash (by usual method), (3) Total nitrogen (by Kjeldahl's micro-method), (4) Protein nitrogen

(by Stuzer's method), (5) Non-protein-nitrogen, (6) Amino-nitrogen (by Van Slyke's method), (7) Reducing sugar (Somogyi's colorimetric method[39,40]), (8) Water soluble-N (usual method), (9) Hot water soluble-N, (10) Amount of Cu: (It was estimated by a photoelectric colorimeter with the addition of Cupresol's reagent[41]). (11) Amount of Fe: This was estimated by a photoelectric colorimeter with the addition of thioglycolic acid. (12) Detection of free sugar: This was done by the same method as described in the previous paper[13,42]. (13) Detection of free amino acid: Samples of amino acid solutions obtained from crab meat by Neuberg[43] and Fujii's methods[44] were developed by the paper chromatography and revealed by ninhydrin butanol solution. (14) The estimation of the degree of "browning" of canned crab meat: This was done with an A.K.A. photoelectric colorimeter.

4. *Experimental results*

The difference of the chemical components of crab meat as they existed before and after the peeling is shown in Table 7. In Table 8 the relation between the difference of the degrees of "browning" of canned crab meat and that of the chemical components before and after peeling is shown.

As seen in Table 7, in the peeled-crab meat, the amount of water-content was high, but other components, *e.g.* ash, pure protein-N, nonprotein-N, amino-N and reducing sugars were less than in the hard-shell crab meat. On account of those differences of the chemical components, the taste of the former

Table 7. The difference of chemical components between peeled-crab meat and hard-shell crab meat

Items \ Kinds of carcass	Peeled-crab	Hard-shell crab
Water-content (%)	85.9	82.6
Ash (%)	2.54	3.19
Total-N (%)	1.410	1.792
Protein-N (%)	1.004	1.243
Non-protein-N (%)	0.393	0.546
Amino-N (%)	0.298	0.363
Water soluble-N (%)	0.506	0.589
Hot water soluble-N (%)	0.261	0.288
Total amount of reducing sugar (*mg*%)	186.0	284.0
Glucose (*mg*%)	18.4	35.6
Ribose (*mg*%)	trace	37.6
Total amounts of Cu (*mg*%)	10.1	11.9
Total amounts of Fe (*mg*%)	—	5.7
Amount of Cu in hot water extract (*mg*%)	4.37	3.75
Amount of Cu in cold water extract (*mg*%)	6.25	10.0

may be inferior to that of the latter. Difference in the amount of glucose was not seen in the two crab meats, but while the amount of ribose was only barely perceptible in the peeled-crab meat, that in the hard-shell crab meat was about 38 *mg%*. Thus the difference of the amount of ribose was remarkable. Difference of the amounts of Cu and Fe in the two meats was scarcely seen.

Table 8. Quality of the canned crab prepared from the peeled-crab and hard-shell crab by the different boiling methods

Items estimated \ Kinds of crab / Boiling method		Peeled-crab: High temp.	Peeled-crab: Low temp.	Hard-shell crab: High temp.	Hard-shell crab: Low temp.
pH		6.8	6.6	6.0	6.2
V.B.-N (*mg%*)		25.5	18.0	30.5	22.4
NH_2-N (*mg%*)		124.5	142.9	166.5	197.3
Reducing sugar (*mg%*)	in meat	24.2	31.0	24.3	29.5
	in juice	27.6	25.0	21.0	18.5
Amount of Cu (*mg%*)	in meat	5.0	3.4	4.8	3.4
	in juice	1.6	1.8	2.7	—
Amount of Fe in juice (*mg%*)		4.5	—	6.8	—
Degree of browning (reflectancy, %)		62.5	61.0	43.5	41.0

According to the results obtained for detection of the kinds of amino acids in the two crab meats by paper chromatography, in the hard-shell crab meat, cystine, lysine, arginine, histidine, glycine, phenylalanine and leucine were detected, but in the peeled-crab meat histidine and arginine were not detected.

It was stated in the previous paper[13,14] that the degree of "browning" of the canned crab meat is effected by the amounts of amino acids and reducing sugars or by the concentration of metallic ions of Cu or Fe. From the results obtained as described above, "browning" of the canned crab which was prepared from peeled-crab meat is considered to be less probable than in that from hard-shell crab.

In fact, as seen in Table 8, "browning" of the canned peeled-crab meat was less than that of the hard-shell crab meat due to either "high temperature boiling method" or "low temperature boiling method".

Thus the reason why the canned peeled-crab meat has less "browning" than the canned hard-shell crab meat was interpreted from the fact that the chemical components concerned with "browning" were less in the former than in the latter.

V. THE RELATION BETWEEN THE "BROWNING" OF CANNED CRAB AND THE STORING METHODS OF RAW CRAB CARCASS WITHOUT CARAPACE

From those results in the previous article III, it seems reasonable that, if the accumulation of the various chemical components which cause "browning" as mentioned above could be controlled by some method, and also, if the chemical components could be induced to flow out from the meat as a water extract before the filling process, the "browning" of the canned meat could hardly occur. In view of these ideas, the next experiment was concerned with estimations of the progressive variation of chemical components in the carcass stored for 48 hours under various conditions. Measurements were made of the degree of the "browning" of the canned meat prepared from carcass which had been stored as mentioned above.

1. *Samples used*

After being landed, fresh raw crab carcass was selected organoleptically in order to secure the meat with the same freshness; half portions of each crab body served to form groups of samples.

2. *Preparation of sample cans*

Each group of the sample (carcass) was stored for 48 hours at 0°～3°C under various conditions as shown in the 2nd column of Table 9. That is to say, the carcass was stored in pure ice, ice contained C.T.C. (aureomycine) or ice water containing other various chemical compounds, or ice water, of which pH was adjusted to 4, 5, 6, 7, 8 value by adding N/10 HCl or N/10 NaOH solutions. During storage, progressive variation of chemical components of each sample was separately estimated from aspects of the items below.

Each group of the meat of carcass stored for 48 hours under various conditions as mentioned above was separately boiled at a temperature of 100°C for 18 minutes, and then the meat was removed from the crust. As the washing process, the meat removed from the crust was soaked in water for 5 minutes; then it was processed into canned crab in the usual way. After 50 days storage, the cans were opened and the degree of "browning" of their contents was estimated for the meat from aspects of the items below.

3. *Items estimated and the method of estimation*

As done in the previous paper[13], volatile basic nitrogen, amino nitrogen, and reducing sugar were estimated together with the degree of "browning" of the content of the can.

4. *Results*

The results of these estimations obtained from the crab carcass stored for 48 hours under various conditions are shown in Tables 9 and 10. As seen in the 3rd and 4th columns of Table 9, the variation of amounts of volatile

Table 9. Variation of the amounts of chemical components in the raw crab meat with crust which was stored under various conditions

Sample No.	Conditions of storing the meat \ Items; Time of leaving (hrs.)	0				48			
		pH	V. B.-N (*mg%*)	Amino-N (*mg%*)	Reducing sugar (*mg%*)	pH	V. B.-N (*mg%*)	Amino-N (*mg%*)	Reducing sugar (*mg%*)
1	With crushed ice	6.1	8.65	185.9	28.0	6.5	16.3	163.4	23.5
2	With crushed ice containing 5 *p.p.m.* of C.T.C.	〃	〃	〃	〃	6.3	10.1	173.5	33.0
3	In ice water (pH 5.7)	〃	〃	〃	〃	6.0	7.0	163.4	21.0
4	In ice water containing 5 *p.p.m.* of C. T. C.	〃	9.65	186.0	〃	6.4	5.7	263.0	38.0
5	In ice water containing 0.05% sodium bisulfite	6.2	〃	〃	〃	〃	7.1	137.7	20.0
6	In ice water containing 0.3% of sodium bisulfite (pH 5.6)	〃	10.1	185.8	19.6	6.7	9.6	83.7	8.0
7	In ice water containing 0.3% citric acid (pH 5.0)	〃	〃	〃	〃	5.9	8.4	119.9	22.0
8	In ice water containing 0.3% of boric acid (pH 4.0)	〃	〃	〃	〃	6.0	3.1	83.7	〃
9	In ice water containing 0.2% of sodium citrate (pH 7.8)	〃	10.0	〃	〃	7.0	6.6	〃	18.0
10	In ice water containing 0.2% of borax (pH 8.8)	〃	10.1	〃	〃	7.6	〃	136.7	16.0
11	In ice water of which pH was 4.0	〃	〃	〃	〃	6.0	3.8	77.6	18.0
12	In ice water of which pH was 5.0	〃	〃	〃	〃	6.4	〃	141.3	20.0
13	In ice water of which pH was 6.0	〃	〃	〃	〃	〃	6.2	75.4	18.0
14	In ice water of which pH was 7.0	〃	〃	〃	〃	〃	7.7	102.8	16.0
15	In ice water of which pH was 8.0	〃	〃	〃	〃	7.4	3.5	128.2	8.0

Table 10. Amount of chemical components flowed out from the raw crab meat which was stored under various conditions

Conditions of storing the meat			Amount of chemical components flowed out from 100*g* of the meat	
Temperature of leaving (°C)	Time of left alone	Properties of water in which the meat was stored	Total-N %	Cu (*mg*%)
3~0	48	0.3% sodium bisulfite	0.234	24.0
〃	〃	0.3% citric acid	0.109	0.7
〃	〃	0.3% boric acid	0.201	6.0
〃	〃	0.2% sodium citrate	0.163	18.0
〃	〃	0.2% borax	0.132	18.0
〃	〃	pH 4.0	0.213	16.0
〃	〃	pH 5.0	0.168	0.3
〃	〃	pH 6.0	0.245	24.0
〃	〃	pH 7.0	0.229	17.0
〃	〃	pH 8.0	0.198	16.0

basic nitrogen, amino nitrogen, and reducing sugar in the stored meat were affected by the storing condition. Quantitative variations of amino nitrogen and reducing sugar in the meat stored with crushed ice were not remarkable, but those amounts in the meat stored in ice water were reduced in a large quantity.

In the case of the storing of the meat in ice water, the variation of the amount of the chemical components were remarkably affected by the properties of the water in which the meat was stored together with crushed ice. When the meat was stored in ice water, there was remarkably more reduction in the amount of the chemical components in the meat stored in ice water of which pH was above 6.0 or below 4.0 than that of pH 5.0. This tendency is considered as due to the fact that water soluble components in the meat flowed out as water extracts.

As shown in Table 10, the total amount of nitrogen and Cu which flowed out from the meat was affected by the properties of the water in which the meats were stored together with crushed ice. The effect of differences in the conditions of storing the meat on amounts of effluence is observed to be more remarkable in the Cu compounds than in nitrogen compounds.

The results obtained from the canned crab which was prepared from the raw material stored for 48 hours at 0°~3°C under various conditions is shown in Table 11.

As seen in Table 11, the degree of the "browning" of the canned crab meat was affected remarkably by the conditions under which the carcass (raw meat) was stored. The canned meat prepared from the raw materials which

Table 11. Comparison of quality of canned crab prepared from the raw crab meat with crust which was stored under various conditions

Sample No.	Items / Conditions of storing the meat	Browning degree of the canned meat (reflection ratio, %)		pH	V. B.-N (*mg%*)	Amino-N (*mg%*)	Reducing sugar (*mg%*)
		White meat	Whole meat				
1	With crushed ice	41.5	35.5	7.0	15.3	173	20
2	With crushed ice containing 5 *p.p.m.* of C. T. C.	41.8	32.0	6.4	32.5	247	20
3	In ice water	45.5	39.0	6.6	21.3	141	18
4	In ice water containing 5 *p.p.m.* of C. T. C.	35.5	39.0	6.4	27.0	211	22
5	In ice water containing 0.05% sodium bisulfite (pH 5.6)	48.5	37.5	6.8	25.1	109	22
6	In ice water containing 0.3% of sodium bisulfite (pH 5.6)	41.5	29.5	7.0	16.0	96.1	26
7	In ice water containing 0.3% citric acid (pH 5.0)	39.2	35.5	6.8	16.2	83.3	20
8	In ice water containing 0.3% of boric acid (pH 4.0)	45.5	35.5	6.8	8.2	135	26
9	In ice water containing 0.2% of sodium citrate (pH 7.8)	47.5	43.5	6.8	7.9	128	18
10	In ice water containing 0.2% of borax (pH 8.8)	51.0	34.0	6.6	10.9	96.1	20
11	In ice water of which pH was 4.0	45.2	40.2	6.4	21.7	66.6	26
12	In ice water of which pH was 5.0	39.5	33.5	6.6	18.8	135	20
13	In ice water of which pH was 6.0	52.2	42.5	6.6	11.5	135	18
14	In ice water of which pH was 7.0	49.5	40.2	6.8	15.7	96.1	24
15	In ice water of which pH was 8.0	40.5	39.5	6.8	14.5	76.9	—

were stored in ice water adjusted to pH 6 or 7 with the addition of N/10 HCl or N/10 NaOH solutions; or stored in ice water containing 0.2% borax (sodium tetra borate) solution, 0.2% sodium citrate solution, and 0.05% sodium bisulfite solution, was white in color and the "browning" phenomenon did not completely appear. The merchandise value of this canned crab was raised by a rich elasticity and a good taste.

When the canned crab was prepared from the raw meat which was stored under other conditions [*e.g.* stored in ice water containing 5 *p.p.m.* of C.T.C. (aureomycine) solution, 0.3% citric acid solution, or solution adjusted to pH 5.0 respectively] described above, the canned meat showed brown in color and became fragile with poor elasticity; the merchandise value of this canned crab was reduced in contrast with samples prepared from the meat which was stored with crushed ice.

VI. THE AMOUNTS OF CHEMICAL COMPONENTS WHICH FLOWED OUT WHEN RAW CRAB MEAT WAS BOILED DURING THE PROCESSING OF CANNED CRAB

In the previous articles II and V, it was made clear that "browning" of canned crab meat is influenced by the amounts of amino compounds and reducing sugars or by a high concentration of Cu or Fe, therefore by the removing of those components the "browning" can be considerably prevented. Also, it was clarified that the coagulation of water soluble protein in the crab meat, or of haemocyanin in the blood, and the heat contraction of crab meat which have intimate relations with the "browning", are effected by the boiling temperature, pH value and the kind of boiling-water. Therefore, it is supposed that "browning" will be effected by the boiling factors (*e.g.* boiling temperature and kind of boiling-water).

Here, in order to clarify the matter of these boiling factors, the author has estimated the flowing amounts of chemical components concerned with the "browning", when the raw crab meat samples having various degrees of freshness are heated at various temperatures in water having various pH values.

A. The amounts of chemical components flowed out when the raw crab meats having various degrees of freshness were heated at various temperatures

1. *Sample*

The carcasses from which the carapaces were removed were stored in ice in the hold for 12 hours. The meat taken was crushed by a homogenizer and used for sample.

2. *Experimental method*

By storing the crushed raw crab meat at 5°C the samples having various degrees of freshness (7.5, 15.6 and 19.3 *mg%* of V.B.-N, respectively) were

prepared. One hundred *cc* of distilled water was added to 10 *g* each of the raw crab meats placed in large test tubes. Each test tube was heated for 20 minutes in water kept at definite temperatures from 30°C to 100°C in a heating vessel with shaking of the content. After the heating, the volume of the content was made to 100 *cc* by adding water, and filtered. A part of the filtrate was used for the estimation of amounts of total nitrogen, Cu and Fe by the same methods described in the previous article IV. Another part of the filtrate was used for the estimation of amino nitrogen by Van Slyke's method.

3. *Experimental results*

Results obtained are shown in Figs. 2 and 3.

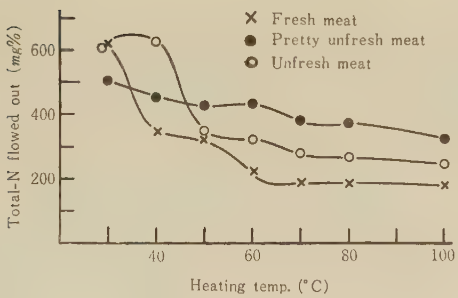


Fig. 2. Amount of total-N flowed out when raw crab meat was heated at various temperatures

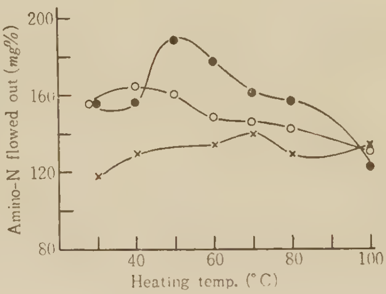


Fig. 3. Amount of amino-N flowed out when raw crab meat was heated at various temperatures (See Fig. 2 as to the different marks)

As seen in Figs. 2 and 3, when fresh raw crab meat was heated, the amount of total-N flowing out was small at a high temperature, but the amount of amino-N was largest when the fresh raw meat was heated at 70°C. As to the relation between the amounts of total-N or amino-N and the degrees of freshness of the raw crab meat, those amounts were large, when the freshness fell slightly, but were small when the freshness fell remarkably. The temperature at which the amount of amino-N which flowed out from the meat showed the maximum was dependent upon the freshness, for examples, it was 70°C for the fresh meat (7.5 *mg%*, V.B.-N), 50°~60°C for the fairly unfresh meat (15.6 *mg%*), and 40°~50°C for the unfresh meat (19.3 *mg%*). Thus as the temperature moved to the lower side, with the falling of the freshness the out-flow was high.

In the results obtained, the reason that the total amount of nitrogen flowing out decreased at about 70°C with the rising of heating temperature, and de-

creased scarcely above 70°C may be due to the heat coagulation of the meat, because the crab meat which consists of various proteins such as myosin, myogen and haemocyanin coagulates completely at 70°~80°C[10].

It has been observed by Shimidu[45,46] or Fujii[47] in fish meat that the out-flowing amount of amino-N was the maximum at 70°C for the fresh meat, but the temperature of the maximum flowing amount moves to the lower side for the fairly unfresh or unfresh meat; as to the relation between the freshness and flowing amount, the amount was large in the fairly unfresh meat but it was small in the unfresh meat. These facts have also been observed by the present author in the studies of dehydration, absorption or heat-contraction in crab meat by heating[38,48]. Those facts may be explained as a cause of the denaturation of meat protein accompanying with the falling of freshness.

From the results obtained, in order to cause chemical components to flow out abundantly, for example, amino compounds concerned with "browning", the fresh raw crab meat material is considered best to be heated by the "high temperature boiling method" (at 100°C), and the unfresh one to be heated by the "low temperature boiling method" (at 60°~70°C).

B. The amounts of chemical components flowed out when the raw crab meat was heated in water having various pH values

1. *Sample*

Samples similar to those described in the previous article were used.

2. *Experimental method*

Ten *g* samples of the meat were put respectively into large test tubes. One hundred *cc* of McIlvain's buffer solution having various pH values, pH 3~8, were added to each tube. Those test tubes were maintained at various temperatures (20°C, 60°C and 100°C) for 20 minutes with shaking. After the cooling, the content of the test tube was made to 100 *cc* by addition of the respective McIlvaine's buffer solution making up a loss of evaporation during heating. After the filtration of the content in the test tubes, the filtrate was employed for the estimation of total-N, amino-N, Cu, Fe and reducing sugar.

3. *Experimental results*

Results obtained are shown in Figs. 4~8.

As seen in Fig. 4, the flowing amount of total-N at 20°C was effected by the pH values of heating-water, that is to say, in pH 5 the amount was minimum, where as the amounts to the acidic or the alkaline sides from pH 5 were larger than that at pH 5. On the other hand, in heating at 60°C or 100°C the effect upon the flowing amounts was not remarkable.

Strictly speaking, with the movement of pH value to the alkaline side the flowing amount became comparatively large. As seen in Figs. 5~8, the flowing amounts of amino-N, reducing sugar, Cu and Fe were affected by pH value of the heating water and heating temperature. At high temperature (100°C) the amounts were large on the acidic side of pH 6, and were small on the alkaline

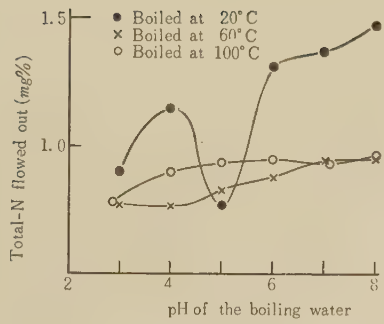


Fig. 4

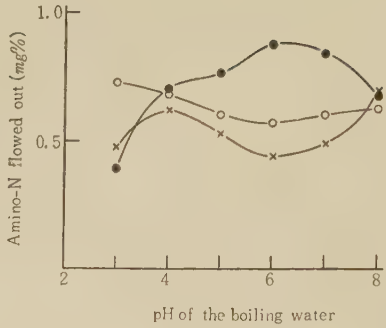


Fig. 5

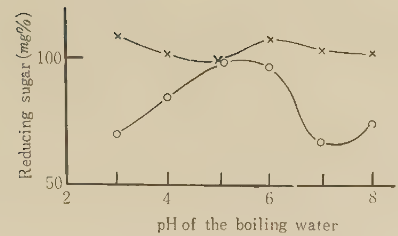


Fig. 6

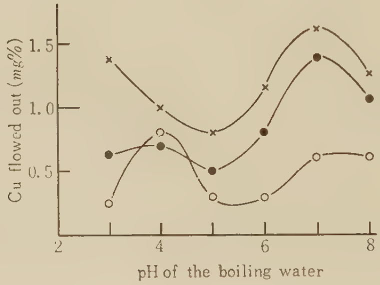


Fig. 7

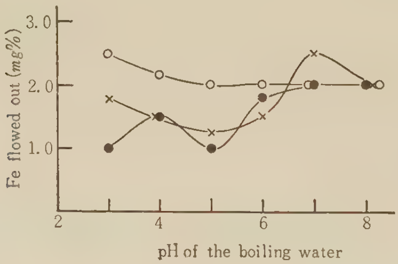


Fig. 8

Fig. 4. Amount of total-N flowed out when the crab meat was heated in water having various pH values

Fig. 5. Amount of amino-N flowed out when the crab meat was heated in water having various pH values (See Fig. 4 as to the different marks)

Fig. 6. Amount of reducing sugar flowed out when the crab meat was heated in water having various pH values (See Fig. 4 as to the different marks)

Fig. 7. Amount of Cu flowed out when the crab meat was heated in water having various pH values (See Fig. 4 as to the different marks)

Fig. 8. Amount of Fe flowed out when the crab meat was heated in water having various pH values (See Fig. 4 as to the different marks)

side of the same pH value. On the contrary, at 20°C or 60°C the flowing amounts of amino-N and reducing sugar were small on the acidic side of pH 6, and large on the alkaline side of the same pH value. But in the results described above, at 20°C or 60°C of heating temperature, the flowing amounts were small at pH 5. This pH value has been considered to be the isoelectric point of *Paralithodes camtschatica* meat protein as observed from the swelling and dehydration or heat contraction phenomenon of the crab meat by heating[38,48].

At 60°C of heating, myosin of which the coagulating temperature are 35°~40°C coagulates completely, but myogen of which the coagulating temperature is about 70°C does not coagulate yet. Therefore, the flowing amounts are considered to be small in the range of the isoelectric reaction at temperature of 60°C.

The fact that the amounts of chemical components flowing out from the crab meat become large outside of a boundary of the isoelectric reaction, agrees with the observation of the swellings of the meat at acidic or alkaline sides. This agrees with Okada's consideration[49] that the swelling phenomenon is one of the proceeding steps of the dissolution of the meat.

Further, the fact that the flowing amounts of chemical components were considered to be large on the acidic side below pH 6 as the results of the heating at 100°C, or contrarily on the alkaline side at 60°C, will be explained as due to the reason that at 100°C the amounts become large owing to the sum of factors of mechanical pressure brough about by the complete coagulation of muscle protein and heat-contraction of the muscular fiber of the meat and by the increase of the amount of soluble matter caused by the acid swelling of the meat. On the other hand, at 60°C the solubility of a part of the meat protein which has not been coagulated yet increases on the alkaline side.

VII. RELATION BETWEEN THE "BROWNING" OF CANNED CRAB AND BOILING METHODS OR KINDS OF BOILING WATER

In this experiment the author tried to find if any relation exists between the degree of "browning" of the canned crab meat and the various properties of the boiling water in which the corresponding raw meat materials were respectively cooked under conditions as described below.

A. The relation between the browning of the canned meat crab and the conditions of boiling of meat with crust

1. *Samples used*

The carcass which were treated by usual method was stored in an ice box at a temperature of 0°~3°C for 78~88 hours. After storage, the carcass was divided into half bodies as lots of samples, and the sample of each lot was boiled under the various conditions as described below.

Table 12. Various conditions in the boiling of the raw crab meat with crust

Sample No.	Conditions of boiling the meat with crust					Conditions of boiling the meat which was removed from crust				
	Temperature of boiling water (°C)	Time of boiling the meat (min.)	Properties of boiling water			Temperature of boiling water (°C)	Time of boiling the meat (min.)	Properties of the boiling water		
			pH	Added compound	Amounts added (%)			pH	Added compound	Amounts added (%)
1	100	18	5.7	Blank (fresh water)	—	—	—	—	—	—
2	〃	〃	5.5	Boric acid	0.1	—	—	—	—	—
3	〃	〃	5.0	Citric acid	〃	—	—	—	—	—
4	〃	〃	8.0	Borax	〃	—	—	—	—	—
5	〃	〃	7.0	Sodium citrate	〃	—	—	—	—	—
6	〃	〃	6.8	Sodium citrate	—	—	—	—	—	—
7	60	10	5.7	Blank (fresh water)	—	100	3	5.7	Blank (fresh water)	—
8	〃	〃	4.5	Hydrochloric acid	Small amount	〃	〃	7.5	Sodium hydroxide	Small amount
9	〃	〃	7.5	Sodium hydroxide	〃	〃	〃	4.5	Hydrochloric acid	〃
10	〃	〃	5.5	Boric acid	0.1	〃	〃	8.0	Borax	0.1
11	〃	〃	8.0	Borax	〃	〃	〃	4.8	Boric acid	〃
12	〃	〃	4.8	Citric acid	〃	〃	〃	7.0	Sodium citrate	〃
13	〃	〃	7.0	Sodium citrate	〃	〃	〃	4.8	Citric acid	〃
14	〃	〃	6.8	Sodium bisulfite	0.3	〃	〃	6.8	Sodium bisulfite	0.3
15	〃	〃	6.8	Sodium bisulfite	〃	〃	〃	5.7	Fresh water	—
16	〃	〃	5.7	Fresh water	—	〃	〃	6.8	Sodium bisulfite	0.3

2. *Preparation of sample cans*

Into five *l* of fresh water in which various chemical compounds were added as shown in Table 12, each lot of the carcass was placed separately and boiled by two different methods; one method was boiling the carcass with crust at 100°C for 18 minutes; the other method was boiling the carcass at 60°C for 10 minutes (under the condition in which heamocyanin protein in the meat is not coagulated).

After removal of meat from the crust which was boiled at 100°C, the meat was washed separately with fresh water and then processed into canned crab in the usual way. However, the meat boiled at 60°C was taken from the crust with a compress roll and then the meat removed from the crust was boiled at 100°C for 3 minutes.

Manufactured canned crab was brought into the laboratory and stored for 60 days at room temperature. After opening the cans, the author estimated the relationship between the degree of "browning" of their contents and the conditions of boiling process from the aspects of items below.

3. *Items estimated and estimation method*

As in the previous experiment, estimations were made of pH, volatile basic nitrogen, total nitrogen, protein nitrogen, amino nitrogen and reducing sugar together with the degree of "browning" of the content. Sometimes separate estimates were made for meat and juice.

4. *Results*

In Table 13 are tabulated the estimates made of values of the properties of the canned crab prepared from the meat which was boiled under the various conditions shown in Table 12. The "browning" degree of the canned crab, as shown in Table 13, was influenced remarkably by the condition of the boiling process. When the raw meat with crust was boiled completely for 18 minutes in the boiling water (100°C) with pH adjusted to the acidic side (about pH 5.0), and was then manufactured into canned meat, the "browning" of the finished product was slight and the meat showed no more than a pale yellow in color. Canned meat prepared from the corresponding raw material boiled in water of pH values above 6.6, especially in the alkaline region, exhibited a deep brown color.

In the processing of canned crab by the "low temperature boiling method" when use was made of boiling water above pH 6.5, and the boiled crab meat without crust was reboiled in boiling water whose pH value is below 6.5, the canned crab product showed white in color. Thus it was ascertained that the occurrence of the "browning" reaction in canned crab may be prevented. On the contrary, even if the meat was taken from the crust by the boiling process as done above, when the removed meat was reboiled in boiling water with a pH value on the alkaline side, the degree of "browning" of the canned meat was obviously increased and the meat showed deep brown in color. The browning

Table 13. Comparison of the quality of canned crab prepared from raw crab meat which was boiled under various conditions

Sample No.	Browning degree (reflection ratio, %)		pH of meat	V.B.-N in meat ($mg\%$)	Total-N in meat (%)	Protein-N in meat (%)	Amino-N		Reducing sugar	
	White meat	Whole meat					in meat ($mg\%$)	in juice ($mg\%$)	in meat ($mg\%$)	in juice ($mg\%$)
1	40.5	30.0	6.6	30.3	3.42	2.89	172	239	18	26
2	36.5	28.5	6.8	45.9	3.34	2.84	211	258	20	18
3	42.5	31.5	6.8	23.8	2.99	2.41	179	214	26	24
4	29.5	26.0	6.4	37.9	3.23	2.57	211	307	22	24
5	22.5	20.5	6.4	36.0	3.21	2.44	200	261	22	32
6	29.5	26.5	6.5	30.9	3.52	3.08	224	297	15	32
7	44.0	34.5	6.8	31.4	3.06	2.84	160	164	22	26
8	39.5	34.5	6.6	25.7	3.28	2.76	211	250	26	22
9	41.5	39.0	6.8	22.7	3.30	2.89	135	177	20	22
10	36.5	34.0	6.6	27.3	3.17	3.27	179	159	18	20
11	49.5	39.5	6.6	25.9	3.19	2.80	166	230	24	22
12	36.0	29.0	6.3	30.3	3.31	2.87	182	280	20	18
13	46.5	31.5	6.6	26.8	3.07	2.89	141	188	18	24
14	28.0	28.0	6.4	36.9	3.55	2.89	196	282	20	15
15	43.5	39.0	6.6	31.4	3.07	2.97	186	220	22	20
16	33.0	29.5	6.6	37.7	3.28	2.77	254	267	20	18

of the canned meat was independent from the total amount of nitrogen or protein nitrogen.

It was clear that the amount of free amino nitrogen in the browned canned crab was comparatively larger than that in normal canned, and pH value of the browned meat was smaller than that in the normal can. Also, as ascertained in the previous paper[13], the browned canned crab meat was less elastic than the normal meat, and the muscle cellular group began to shrink. The browned canned crab meat has a caramel smell and a poor merchandise value in taste or other appearances. On the contrary, the normal canned crab has a rich merchandise value from many aspects.

B. Degree of the "browning" of canned crab prepared from raw materials boiled in water containing complex salt-making reagent in various concentrations

1. *Selection of complex salt-making reagents*

As done in the previous paper[14], white meat was removed from crust of the fresh raw material (carcass) with knife and used as samples. Various kinds of complex salt-making reagents, such as *curafos, calgon* (a kind of polyphosphate; Japan Organo Co. Ltd.), sodium-metaphosphate, and ethylendiaminetetraacetate (E.D.T.A) were used to convert the metals in the crab meat to the corresponding water soluble complex salts of these metals.

(1) *Experimental method*

Erlenmeyer flasks in which 20 *g* each of samples (raw meat) were put, were divided into two groups. To these Erlenmeyer flasks, 20 *cc* of solutions containing 1% of the weight of the reagents mentioned above, were separately added. Then one group of the flasks was heated for 18 minutes in a water bath kept at 100°C, while the flasks of the other group were heated for 10 minutes in the water bath at 60°C. After the washing of the meat with water, these flasks were plugged with cotton and heated at 6 pounds pressure for 80 minutes. After cooling, the degrees of "browning" of the processed meat samples were measured as recorded in the previous paper[13]. Determinations of Cu and Fe were carried out with diethanol amine[41] and thioglycolic acid, respectively[50].

(2) *Result*

As seen in Table 14, amounts of Cu and Fe in the meat were noticeably influenced with the conditions of the boiling process. The difference was considered to be caused by the factors that the metals in the crab meat flowed easily in the boiling water as the complex salts in water soluble state which were formed between the metals in the crab meat and the reagents added in the boiling water. The degree of "browning" was also affected by the kinds of reagents which were added into the boiling water. The meat which was boiled in the solution containing *curafos* showed white in color, and it contained a little amount of the metals which will cause "browning".

From these results, it was considered that the addition of *curafos* into the boiling water is available for the preventing of the "browning" of the canned

crab meat.

Table 14. Degree of "browning" of the crab meat which was boiled in water containing various kinds of salt-making reagents

Boiling method	Kinds of boiling water	Degree of browning (reflection ratio, %)	Amount of Cu (*mg*%)	Amount of Fe (*mg*%)
Low temperature method	0.1% *calgon F. G.*	36.1	2.00	2.2
	0.1% *curafos*	43.9	1.50	3.0
	0.1% Sodium metaphosphate	35.0	1.88	3.7
	0.1% E. D. T. A.	33.1	2.00	3.2
	Distilled water	37.0	2.38	4.0
High temperature method	0.1% *calgon F. G.*	39.5	2.32	3.4
	0.1% *curafos*	48.6	1.75	2.7
	0.1% sodium metaphosphate	41.8	2.75	3.2
	0.1% E. D. T. A.	51.0	2.25	3.4
	Distilled water	42.6	3.50	—

2. *The "browning" of the canned crab meat which has been boiled in water with added complex salt-making reagent and then processed*

(1) *Sample used*

The crab carcasses were stored in an ice box at a temperature of 0°~3°C for 48 hours. The raw crab meat contained in the crust was 6.2 in pH value and the amount of volatile basic nitrogen was 11.0 *mg*%. Those carcasses were employed as samples.

(2) *Preparation of sample cans*

Each carcass was divided into half the portion, and those divided portions were classified into two groups. The carcasses of one group were separately put into various kinds of solutions as described below and boiled at a temperature of 100°C for 18 minutes. After removal of the meat from the crust, the meat alone was soaked separately in fresh water for 2 minutes, and processed into cans.

The carcasses of the other group were separately put into portions of each of various solutions as described below and boiled at a temperature of 60°C for 10 minutes. After removal of the meat from the crust by a compressor roll, the meat was soaked in fresh water for 2 minutes. The soaked meat was boiled in water, kept at 100°C for 3 minutes and processed in the usual way.

After cooling, these cans were brought into the laboratory and stored for three months. They were then opened and the effect of boiling water containing the complex salt-making reagent on the "browning" of the canned meat was estimated from the view point of items below.

(3) *Items estimated and the method*

As done in the previous experiment[13], estimations were made of pH, volatile basic nitrogen, amino nitrogen and reducing sugar together with that of the "browning" degree of the canned meat.

(4) *Result*

In Tables 15 and 16 are shown the estimates made regarding the canned meat prepared from the material boiled in various kinds of water to which different amounts of *curafos* were added separately.

As seen in Tables 15 and 16, the canned meat prepared from the meat with crust (carcass) removed from carapace which was boiled in water or sea water containing *curafos*, showed white in color; on the contrary, in the canned meat prepared for a comparative purpose to the meat boiled in water without *curafos* "browning" was observed.

The effect of boiling water containing *curafos* on the "browning" of the canned meat was influenced by the boiling methods ("high-" or "low-temperature boiling method"). When the canned crab was prepared from the raw meat which was boiled at different temperatures of 60°C or 100°C in the water with *curafos*, the former showed whiter in color than the latter meat. However, in the comparative cans prepared from the meat boiled without *curafos*, the degree of "browning" was remarkably more advanced in the canned meat manufactured from that boiled by the "low temperature boiling method" than that boiled by the "high temperature boiling method".

The degree of the "browning" of the canned meat which was prepared from the raw meat boiled with different sorts of water such as fresh water and sea water was not influenced by the kinds of water used; however, the taste or

Table 15. Qualities of the canned crab prepared from meat boiled by the "high temperature boiling method" in water containing *curafos* (a complex salt-making reagent)

Items / Sample No.	H-1	H-2	H-3	H-4	H-5	H-6
Kinds of boiling water	Sea water	Fresh water	Sea water	Fresh water	Sea water	Fresh water
Conc. of *curafos* (%)	0	0	0.1	0.1	0.5	0.5
pH	6.56	6.55	6.15	6.30	6.65	6.50
V.B.-N (*mg%*)	29.7	29.9	27.8	29.9	31.3	29.9
Amino-N (*mg%*)	181.7	156.4	179.1	169.1	163.4	153.1
Reducing sugar (*mg%*)	20.0	20.0	19.5	15.0	15.0	15.0
Browning degree of the meat (reflection ratio, %)	44.5	45.5	50.9	51.5	52.5	52.5
Organo-leptic test: Taste	Good	Good	Good	Good	Good	Good
Organo-leptic test: Elasticity	Poor	Poor	Rich	Poor	Rich	Rich
Organo-leptic test: Formation of $MgNH_4PO_4 \cdot 6H_2O$ crystal	Many	Many	Few	Few	Few	Few

Table 16. Qualities of the canned crab prepared from meat boiled by the "low temperature boiling method" in water containing *curafos*

Items \ Sample No.		L-1	L-2	L-1	L-5	L-5	L-6
Kinds of boiling water		Sea water	Fresh water	Sea water	Fresh water	Sea water	Fresh water
Conc. of *curafos* (%)		0	0	0.1	0.1	0.5	0.5
pH		5.55	5.85	6.75	5.95	6.10	6.20
V. B.-N (*mg%*)		27.3	29.9	27.3	29.9	30.1	33.1
Amino-N (*mg%*)		145.1	137.7	130.5	109.4	120.8	97.0
Reducing sugar (*mg%*)		22.0	20.0	15.0	15.0	17.5	15.0
Browning degree of the meat (reflection ratio, %)		42.5	43.0	51.5	53.5	56.4	55.5
Oragno-leptic test	Taste	Good	Bad	Good	Bad	Good	Good
	Elasticity	Poor	Poor	Rich	Poor	Rich	Rich
	Formation of $MgNH_4PO_4 \cdot 6H_2O$ crystal	Very much	Very much	Non	Non	Non	Non

elasticity of the canned meat was remarkably influenced by the kinds of boiling water. The canned meat prepared by boiling with sea water containing *curafos* had a good taste and rich elasticity in comparison to the other samples of canned meat.

From these results, it was ascertained that the raw meat with crust had better be boiled with sea water containing 0.1~0.5% *curafos*.

VIII. "BROWNING" OF CANNED CRAB WHICH WAS PROCESSED FROM MEAT HEATED BY "LOW TEMPERATURE BOILING METHOD"

It is especially said of the floating-cannery where canned crab meat is processed by "low temperature boiling method", that "browning" is observed in cans made even from fresh raw crab meat. On this point the author has investigated the following problems in order to employ the "low temperature boiling method" for processing canned crab of good quality which has no "browning" from fresh raw crab meat.

A. Relation between "browning" of canned crab and the freshness of raw crab of which carapace removed

1. *Sample and method*

The crab carcasses were left at room temperature and were made into samples having various freshness. Each sample of carcasses having the same freshness was divided into two parts: one part was processed by "high temperature boiling method", and the other by "low temperature boiling method". The processing was carried out at 109°C (5.5 pounds pressure) for 80 minutes.

The content of the cans which were opened after two months was studied as to the relation of "browning" to the freshness of the raw crab meat and boiling methods. The items estimated were the same as in the previous article.

2. *Results*

Results obtained are shown in Table 17.

As seen in Table 17, the degree of "browning" of the canned crab meat which was processed by the "high temperature boiling method" increased proportionally with the falling of the freshness of the raw material.

Table 17. Qualities of canned crab meats which were prepared by various boiling methods from the raw crab meats having various degrees of freshness

Degree of freshness	Qualities of raw crab meat		Methods of boiling the meat	Qualities of canned crab meats			
	pH	V.B.-N (*mg%*)		pH	V.B.-N (*mg%*)	Amino nitrogen (*mg%*)	Degree of browning (reflection ratio, %)
Very good	6.4	6.28	High	6.0	21.0	149	50.0
			Low	6.6	20.8	206	39.0
Fairly unfresh	6.0	17.1	High	6.4	28.6	241	39.0
			Low	6.8	25.9	155	40.5
Unfresh	6.8	37.0	High	6.6	34.6	125	33.5
			Low	6.8	25.5	195	37.5

On the other hand, the degree for the cans which were processed by the "low temperature boiling method" did not increase proportionally.

It is considered that when fresh raw meat is employed, the degree of "browning" of canned crab meat which is processed by "low temperature boiling method" is larger than that which is processed by "high temperature boiling method". On the contrary, when unfresh raw meat was employed, the degree of the former is less than that of the latter. The results agree with the Japanese Canned Food Inspection Association's estimation[12].

The reason for this may be explained from the fact deduced in the previous article (VI) that the heating temperature at which the maximum amount of chemical components flowing from the meat was obtained, was dependent principally upon the freshness; 70°~80°C above for the fresh meat, 50°~60°C for fairly unfresh meat.

B. Relation between the "browning" of canned crab meat and boiling temperature

The author has tried to make canned crab meat which has no "browning" by investigating the relation between "browning" and boiling temperature.

1. *Heat-penetration in crab meat during the heating*

The optimum temperature for action of enzymes in raw crab meat was observed to be 45°~55°C, so that unless the meat is heated at 70°~80°C, the

action of the enzymes may not be destroyed[51]. If the temperature of the center of raw crab meat rises too slowly to pass the range of the optimum temperature for the autolytic action of enzymes, the meat will be decomposed by the enzymes to yield the chemical components concerned with "browning". It is important to ascertain the heat-penetration of raw crab meat during the heating at various temperatures. The author has tried to estimate the heat-penetrations in leg meats which were heated at 60°, 70°, 80° and 100°C respectively.

(1) *Experimental method*

Holes were made on the surface of the crust of crab leg (1st and 2nd legs) with a gimlet. The end of a thermocouple was inserted in the center of the leg meat. The lef meat with crust was heated in sea water kept at 60°, 70°, 80° and 100°C each for 10 minutes. The temperatures in the center of the meat were estimated by a potentiometer for a definite interval of time. The heat-penetrations were charted on a section paper as heating temperature–time curve.

(2) *Results*

The curves of heating temperature–time are shown in Fig. 9. The next calculation was made of the time required to pass the range of optimum temperature (45°~55°C) of action of enzymes with obtained data shown in Table 18.

From the results obtained above, it is seen to be important to heat the raw crab meat above 70°~80°C to destroy the autolytic action of enzymes, and to prevent the decomposition of the crab meat.

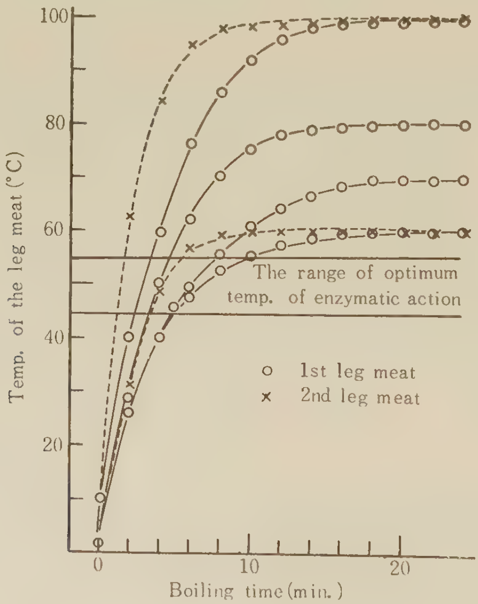


Fig. 9. Changes of temperature of the leg meat during the boiling in water having various temperatures

Table 18. The time required to pass the range of optimum temperature of enzymatic action in the center of the leg meat when the meat was boiled in water having various temperatures

Parts of leg meat \ Temp. of boiling water (°C)	60	70	80	100
1st leg meat	4.0 (min.)	3.6	1.2	0.8
2nd leg meat	1.6	1.2	0.6	0.4

2. *Degrees of "browning" of canned crab meat which were processed after heating at various temperatures*

In this paragraph the author reports study on the relation between the degrees of "browning" and the respective canned meat of which the raw materials were heated at various temperatures by the "low temperature boiling method" and then processed.

(1) *Sample*

The samples were the same as those in section 1 in this article. The surface skin of the meat was removed and only white meat was employed. The white crab meat was stuffed into a collodion tube (3.5 *cm* in dia., 7 *cm* in length) in order to make a similar size and weight of crab leg meat. The amount of V.B.-N in the sample of the white meat was 8.8 *mg%*, amino-N was 449 *mg%*, reducing sugar was 21.4 *mg%* and pH value was 6.2.

Each sample meat which was stuffed into a collodion tube was heated for 10 minutes in sea water at 60°, 70°, 80°, 90° and 100°C, respectively. After the heating, the collodion tube was torn off from the sample meat and the meat was washed in running water for 2 minutes. After the cooled meat had been left to drain for 10 minutes, the meat was heated in sea water of 100°C for 3 minutes to harden it (hardening boiling). A part of the heated meat was employed for the chemical analysis, and the other part was packed into ¼ pound cans.

The cans were processed at 109°C (5.5 pounds pressure) for 70 minutes. After cooling, they were stored for one month at room temperature. After the opening of the sample cans, each content was divided into two parts: flesh and juice parts. Those parts were employed for the determination of "browning" and for chemical analysis.

(2) *Estimation items and method*

Items estimated and methods employed for the boiled meat and canned meat were the same as those in the previous articles III and V.

(3) *Results*

Results obtained concerning the boiled meat are shown in Table 19. Results concerning the canned meat are shown in Table 20.

As seen in Table 19, there were differences in the amounts of the chemical components in the meats which were heated at various temperatures. The

amounts of amino-N and reducing sugar were minimum in the meat heated at 80°C. The pH value of the heated meat decreased with the rising of the temperature. As seen in Table 20, "browning" of canned crab meat which was processed from fresh raw crab was influenced remarkably by the temperature of the heating of raw crab before the processing.

Table 19. Analytical results of the boiled crab meats which were boiled at various temperatures

Temp. of the boiling water (°C)	60	70	80	90	100
Time of boiling the meat (min.)	10	10	10	10	18
pH	6.6	6.6	6.4	6.2	6.1
V.B.-N (*mg%*)	16.2	18.6	22.5	18.9	10.2
Amino-N (*mg%*)	422	401	396	348	275
Reducing sugar (*mg%*)	22.2	22.0	17.2	17.6	25.0

Table 20. Qualities of canned crab meats which were processed from the crab meats boiled at various temperatures

Temp. of boiling water (°C)		60	70	80	90	100
Time of boiling the meat (min.)		10	10	10	10	18
pH		6.4	6.5	6.4	6.2	—
V.B.-N (*mg%*)		33.4	28.6	22.6	27.3	26.8
Amino-N (*mg%*)	in meat	378	330	308	336	328
	in juice	463	403	583	500	438
Reducing sugar (*mg%*)	in meat	24.8	21.1	18.4	20.6	20.9
	in juice	13.2	21.0	27.6	27.6	23.0
Cu (*mg%*)	in meat	2.0	1.8	2.0	2.1	2.2
	in juice	2.5	1.2	0.5	0.8	1.2
Fe in meat (*mg%*)		4.2	4.0	4.2	4.2	5.6
Degree of browning (reflection ratio, %)		40.8	43.2	48.4	48.0	48.2

The canned crab meat which was processed from the crab meat subjected above 80°C was white, and did not change to brown. On the other hand, the canned meat which was processed from the crab meat heated below 70°C was visibly brown. It was clarified that there exist the following relations between the degree of "browning" of canned crab meat and the amounts of chemical components in the content of cans: (1) The pH value of browned canned crab meat decreases much more remarkably than that of normal canned crab meat after the processing. (2) The amounts of free amino-N and reducing sugar in the browned meat are more than those of the normal canned meat. (3) On the contrary, the amounts of free amino-N and reducing sugar in the juice of browned canned crab are less than those of the normal canned crab. (4) There is no significant relation between "browning" and the amounts of Cu and Fe in the canned meat. (5) There is close relation between "browning" and the

amount of Cu in the juice of the canned crab. (6) The browned meat which is projected by ultra-violet light generates fluorescens.

C. The relation between "browning" and the freshness of meat heated at various temperatures

Next the author reports on his study of the relation between "browning" and the freshness of meat which was heated at various temperatures by the "low temperature boiling method", and left for various periods at 10°C.

1. *Preparation of sample cans*

After the crab carcasses were taken to a cannery, they were divided into three groups. One group (with crust) was heated at 60°C for 10 minutes. Another group was heated at 80°C for 10 minutes. The last group was heated at 100°C for 18 minutes. After heating, the crust was separated from the meat. Each meat sample heated at 60°C or 80°C was reheated at 100°C for 3 minutes (hardening boiling) and left at 10°C in order to obtain the heated meat having various degrees of freshness. The meat heated at 100°C was also left at 10°C, and samples having various freshness were prepared. Those heated meats having various freshness were processed as canned crab.

2. *Estimation items and methods*

After the opening of the cans, the content was divided into solid meat and juice. The amounts of V.B.-N, amino-N, reducing sugar, Cu and Fe in meat and juice were estimated; pH value and the degree of "browning" were also

Table 21. Qualities of the canned crab prepared from meat being left for various intervals after the heating by "high-" or "low temperature boiling method"

Temp. of boiling the raw meat	Time of leaving the boiled crab meat (hrs.)	Degree of browning of canned crab meat (reflection ratio, %)	pH	V.B.-N (*mg%*)	Amino-N (*mg%*)		Reducing sugar (*mg%*)		Cu in juice (*mg%*)
					in meat	in juice	in meat	in juice	
60°C	0	40.0	6.5	26.5	147	—	22.0	—	2.0
	20	39.8	6.4	23.5	367	455	24.5	13.0	2.4
	40	37.2	6.0	60.9	787	1003	26.0	2.3	3.0
	60	35.5	6.8	72.2	848	860	20.8	2.0	3.2
80°C	0	49.0	6.5	—	150	—	18.0	—	0.5
	20	48.0	6.4	22.7	316	570	18.5	27.5	0.6
	40	42.6	6.3	42.6	623	990	22.1	15.0	1.8
	60	38.2	6.4	61.2	758	858	22.0	—	2.5
100°C	0	50.5	6.2	24.6	217	—	18.0	—	—
	20	49.5	6.2	26.9	325	429	21.0	23.0	1.2
	40	40.9	6.4	—	328	342	26.5	21.3	2.0
	60	39.8	6.8	59.0	443	—	26.0	—	2.2

ascertained.

3. *Results*

The changes of the chemical components and the comparison of "browning" in the canned crab processed from meat having various freshness after the heating by "high-" or "low temperature boiling method" are shown in Table 21.

Considering the changes of the chemical components, the velocity of the fall in the freshness of the heated meat was remarkably influenced by the heating temperature.

As seen in Table 21, the "browning" of canned crab was influenced remarkably by the temperature of heating and the period of leaving the heated meat before processing.

When the raw crab meat was heated at 80°C, "browning" was less influenced by the freshness than in the case of that which was heated at 60°C. That is to say, when the raw crab meat was heated at about 80°C, being subjected to "low temperature boiling method" and was processed within 20 hours, the canned meat had no "browning". Unless the raw crab meat which was heated at 60°C for 10 minutes was immediately manufactured into the can, the canned meat turned completely brown and became soft, so the whole quality declined.

From the results obtained, when the "low temperature boiling method" is applied to the processing of the canned crab, the heated meat should be subjected to the next processing without being left for a long time. The heating temperature should especially be above 80°C in order to prevent the autolytic action and bacterial action after the heating.

IX. INFLUENCE OF CRAB BLOOD UPON "BROWNING" OF CANNED CRAB MEAT

In this article, the author described his studies on the amounts of chemical components and chemical characteristics of haemocyanin which are concerned with "browning" of canned crab; he also offers some discussion of the relation between blood and the "browning".

A. General chemical composition of crab blood

1. *Sample*

The body fluid which flowed out from the crab meat when the carapace was removed was put into a polyethylene bag, and brought in ice to the laboratory. The body fluid was filtered through gauze. The filtrate was employed as crude blood of crab.

2. *Estimation items and methods*

The general chemical composition was investigated as described previously in article IV.

3. *Results*

The general chemical composition of the crab blood as obtained by the author is shown in Table 22.

Table 22. General chemical composition of crab blood.

Items \ Sample	*Paralithods camtschatica*	*Erimacrus isenbeckii**	*Brevootia tyrannus***
Wate content (%)	91.4	90.5	—
Ash (%)	1.26	—	—
Total-N (%)	1.07	1.01	2.23
Protein-N (%)	0.45	—	0.51
Non-protein-N (%)	0.079	—	0.073
Amino-N (*mg%*)	27.5	—	21.0
Reducing sugar (*mg%*)	92.0	—	90.0
Cu (*mg%*)	5.75	4.53	—
Fe (*mg%*)	3.26	—	41.0

* Kosakabe, I.[10] ** Oya, T.[52]

As seen in Table 22, the chemical components in crab blood which are concerned with "browning" of canned crab meat, *e.g.* amino nitrogen, reducing sugar, Cu, and Fe are generally greater in amount that in the blood of fish[52].

B. Haemocyanin in crab blood

In order to know the relation between "browning" and Cu, the author has studied the characteristics of haemocyanin which was separated from crab blood.

1. *Separation of haemocyanin*

As soon as the carapace was removed from the crab just caught, body fluid which flowed out from the crab body was put into a polyethylen bag and the sealed bag was brought to the laboratory in a thermos bottle with ice and salt.

According to Allison and Cole's method[53], haemocyanin was separated and refined.

2. *Results*

Table 23. The amounts of Cu and total nitrogen in the preparation of haemocyanin

Kinds of crab from which blood was separated	Total-N (% for dry matter)	Cu (% for dry matter)
Hard-shell crab caught at Wakkanai	16.82	0.172
Peeled-crab caught at Wakkanai	16.71	0.163
Hard-shell crab caught at Nemuro	16.63*	0.183*
Peeled-crab caught at Nemuro	16.74*	0.165*

* Kosakabe, I.[10]

The amounts of Cu and the total amount of nitrogen existing in the haemocyanin are shown in Table 23.

As seen in Table 23, the amount of Cu in haemocyanin which was separated from the blood of peeled crab was less than that in the blood of hard-shell crab.

From the results obtained in the present investigation, it can be said that haemocyanin in crab blood is certainly the source of Cu which accelerate the "browning" of canned crab meat.

C. Influence of crab blood upon the "browning" of canned crab meat

The author undertook to observe the influence of crab blood upon "browning" by the following experiments.

1. *Sample*

The crab white meat of which red epidermis was removed was left in running water for 10 minutes in order to remove the blood as completely as possible.

The white meat was crushed and homogenized by a mixer. This homogenized meat was called "white crab meat", and was employed as a sample for the experiment. The amount of amino-N in the white crab meat prepared was 169.4 *mg%*, the amount of reducing sugar was 124.5 *mg%* and the amount of Cu was 1.5 *mg%*. The crab blood added to the white crab meat was obtained by the method described in the previous section A. One-tenth the amount of crab blood or distilled water was added to certain samples of the white crab meat. Then this white meat was homogenized. Cans were filled with meat containing crab blood and meat without crab blood.

2. *Preparation of sample cans*

Collodion tubes (diameter 3.5 *cm*, length 7 *cm*) filled with the white crab meat, with crab blood or without blood, were heated at 100°C for 20 minutes by the high temperature boiling method. After the cooling of each sample, the meat was taken out from the collodion tube, put into a quarter pound can and was processed at 109°C (5.5 pounds pressure) for 80 minutes. After cooling, each canned crab sample obtained was left at room temperature for one month.

3. *Items estimated and methods*

After the opening of sample cans, the contents were divided into two parts; solid meat part and juice part.

As to each part, the degree of "browning", pH value, the amounts of V.B.-N, amino-N, reducing sugar and Cu were estimated by the methods described in article III. The color of the solid meat was estimated by a colorimeter of Nippon Denshoku Co. Ltd.

4. *Results*

The differences between canned white crab meat with crab blood and that without blood is shown in Table 24.

As seen in Table 24, it is clear that there is a difference between canned white crab meat with crab blood and that without blood. That is to say, the

Table 24. Difference between canned white crab meat with crab blood and that without blood

Items estimated \ Kinds of sample		Canned crab meat with blood	Canned crab meat without blood
Degree of browning	of meat (reflection ratio, %)	40.8	50.2
	of juice (transparency, %)	53.0	55.5
Color of meat (Hunter[54])	a	222	201
	a′	110	106
	b	166	127
	L	720	733
pH		5.9	6.0
V. B.-N (*mg%*)		13.2	12.9
Amino-N (*mg%*)	in meat	118.5	121.3
	in juice	135.5	162.6
Reducing sugar (*mg%*)	in meat	125	128
	in juice	10.2	14.5
Cu in juice (*mg%*)		4.5	2.0
Fe in juice (*mg%*)		3.1	2.3

degree of "browning" of canned white crab meat was remarkably influenced by the adding of crab blood. The solid meat or juice of canned crab prepared from white crab meat with crab blood, browned visibly as seen by the naked eye or photoelectric colorimeter[54]. On the other hand, the solid meat or the juice of canned meat prepared from the white crab meat without crab blood, did not change to a brown color.

D. The influence of pH of crab blood on the heat-coagulating temperature of the blood

It has been known that the coagulating temperature of the heat-coagulated protein will be affected by the variation of pH value of the reacting system[10,55,56].

Here the author has tried to rise the coagulating temperature of crab blood above 80°C by changing the pH value of the heating water. First the influence of pH on the coagulating temperature of crab blood was investigated.

1. *Sample*

After the body fluid was filtered, the filtrate was dialyzed against distilled water for two days; next the body fluid was centrifuged. After the globulin proteins precipitated by a centrifugal machine were removed, the upper transparent fiuid was employed as "crab blood".

2. *Experimental method*

Crab blood in which pH was adjusted to 5.6~8.0 by the addition of N/10 HCl or N/10 NaOH solution was put into respective small test tubes. Those small test tubes were heated at definite temperatures. The temperature at which blood protein coagulates and shows white turbidity was estimated. Thus

the variation of the coagulating temperature of crab blood was observed as influenced by the variation of the pH value of the blood.

3. *Results*

Results obtained are shown in Table 25.

Table 25. Coagulating temperature of crab blood having various pH values

pH values of crab blood	5.6	6.2	6.8	7.0	7.5	8.0	8.5
Coagulating temperature of crab bood (°C)	68	69	71	71	74	80	85

As seen in Table 25, the coagulating temperature of crab blood having a small pH value was low, and the temperature was high for the blood having a high pH value. If pH value of the blood was above 8.0 on the alkaline side, the coagulating temperature became more than 80°C. Therefore, even if the crab blood is heated at 80°C, when the blood has been adjusted to the alkaline side of pH 8.0, it does not coagulate, does not lose its liquidity, and flows easily from the crab meat.

E. Relation between degree of decomposition of haemocyanin and "browning"

It has been assumed that the source of Cu dissolved in the juice of canned crab is principally in the haemocyanin of the crab blood, because it has been calrified that there is a large amount of Cu in haemocyanin of crab blood.

If crab meat was put into a can with a definite amount of E.D.T.A. (ethylendiaminetetraacetate) and was processed, the juice of the canned crab meat would become blue. This may be explained by the fact that Cu will dissociate from haemocyanin molecule resultant from the complex action of E.D.T.A. and the complex salt of Cu will dissolve in the juice of canned crab[10].

The combination of Cu atom with those chemical compounds has been known to be influenced by the heating temperature and pH value of heating water[57,58].

Therefore, according to the heating conditions of crab carcasses (such as heating temperature, pH value of heating water) Cu in crab blood will be dissociated during the boiling process or processing and the amount of Cu in the juice of the canned crab may increase to result in acceleration of the "browning".

Here, the author reports his investigation of the relation among the dissociation of Cu and the freshness of crab blood, pH value of heating water and heating temperatures.

1. *The influence of pH value of water on the dissociation of Cu in haemocyanin*

(1) *Sample*

Body fluid which was separated from the crab body when the carapace was removed was rapidly frozen and brought to the laboratory in a thermos bottle. After the defrosting of the frozen body fluid, the fluid was treated according to

Scheme 1, and crude haemocyanin was obtained.

Scheme 1. Method of preparation of crude haemocyanin

Blood (body fluid)
↓ Stirring blood with glass stirrer for 10 minutes
Filtration through gauze or filter paper
→ Filtrate / Residue

Filtrate
↓
Dialysis against distilled water for 2 days at 0°~2°C
↓
Separation of precipitate by centrifuge (3000 *r.p.m.*, 20 min.)
→ Supernatant (crude haemocyanin) / Precipitate (globulin)

(2) *Experimental method*

The collodion bags in which 5 *cc* each of the crude haemocyanin was put, were set in separate large test tubes (60 *cc* capacity) containing 20 *cc* of McIlvain's buffer solution of which pH value was adjusted to 4~8. The amounts of the large test tubes were plugged with rubber stoppers. Each test tube was shaken by an apparatus for one hour. After the shaking each test tube was stored in an ice box of 3°C for 24 hours.

The crude haemocyanin solutions in both collodion bag and buffer solution outside of the collodion bag were separately digested with sulfuric acid and perchloric acid. The amount of Cu in both solutions was estimated by the Cupresol's method[41].

Then measurements were made of the variations of the amount of Cu in the haemocyanin molecule and amount of Cu dissociated from haemocyanin according to the pH value of buffer solutions.

(3) *Results*

The amount of Cu in the hoemocyanin molecule and the amount of Cu dissociated from haemocyanin according to the pH value of buffer solutions are shown in Table 26.

As seen in Table 26, the amounts of Cu inside and outside of the collodion bag differed according to the pH value of the buffer solution in which the collodion bag was immersed.

Crude haemocyanin in the collodion bag coagulated as a blue-white material when the bag was immersed in buffer solutions of pH 4.0 and 4.5. When the pH value of the buffer solution was high, the haemocyanin solution in the collodion bag did not coagulate and showed a transparent blue-yellow color.

Table 26. The amounts of Cu combined with haemocyanin and the amounts dissociated from haemocyanin

pH values of buffer solutions	Amount of Cu outside of collodion bag		Amount of Cu inside of collodion bag		Total amount of Cu in 100 cc of sample (%)
	in 100 cc of sample (%)	Cu outside of bag / Total Cu in sample (%)	in 100 cc of sample (%)	Cu inside of bag / Total Cu in sample (%)	
4.0	4.48	70.2	1.90	29.8	6.38
4.5	2.80	47.4	3.10	52.6	5.90
5.0	1.68	32.4	3.50	67.6	5.18
6.0	1.28	22.9	4.30	77.1	5.58
7.0	1.24	21.9	4.50	78.4	5.74
8.0	1.20	—	—	—	—

The amount of nitrogen dissolved from the inside of the collodion bag into the buffers solution outside of the bag increased and showed 0.54~0.58 *g* per 100 *cc* with the increasing of pH value of the buffer solution. The amount of dissolved nitrogen showed a contrary relation to the amount of Cu dissociated.

From the results obtained, Cu in the haemocyanin molecule is considered to dissociate easily.

2. *The influence of temperature on the dissociation of Cu in haemocyanin*

(1) *Experimental method*

Each collodion bag filled with the crude haemocyanin prepared was placed in a big test tube. Each test tube was heated and shaken at various temperature for 30 minutes.

After the cooling of each big test tube, it was left for 24 hours in an ice box at 3°C. Each collodion bag was taken out from its test tube. The amount of Cu in the solution remaining in the big test tube was estimated.

The surface of each collodion bag was washed with distilled water. Then the bag was put into an Erlenmeyer bottle with 10 *cc* of distilled water and the

Table 27. The amounts of Cu inside or outside of

Temp. of heating the sample (°C)	Amount of Cu outside of collodion bag after heating		Amount of Cu bag after
	in 100 cc of sample (%)	Cu outside of bag / Total Cu in sample (%)	in 100 cc of sample (%)
40	1.32	27.2	0.66
50	1.36	26.8	0.90
60	1.24	—	1.06
70	1.20	24.4	0.78
80	1.17	19.5	0.62
90	1.12	17.7	0.58
100	0.92	14.2	0.54

bottle was plugged with cotton. The bottle was heated at 110°C (6 pounds pressure) for 85 minutes in a small retort under the same conditions as in the processing of cans. After the processing, the amounts of Cu in the crude haemocyanin in the collodion bag and that which has penetrated out from the inside of the collodion bag into the bottle were estimated.

(2) *Results*

The results obtained are shown in Table 27.

As seen in Table 27, the amounts of Cu dissociated from haemocyanin differ according to the heating temperatures.

The fact described can be explained as follows: when the haemocyanin solution is heated below 70°C, it will not be coagulated because the coagulating temperature of haemocyanin is 69°~70°C, and it will become unstable, therefore the Cu in the haemocyanin may easily be dissociated. On the contrary, when the haemocyanin is heated above 80°C, it will rapidly coagulate without the dissociation of Cu.

In the previous article, the present author stated that when crab carcasses were heated below 80°C by "low temperature boiling method" in order to prevent the bluing of canned crab meat or to prepare "boneless" canned crab meat, even if very fresh raw crab was used, the canned meat "browned", and the amount of Cu in the juice of the can became large. From those facts the author has assumed that the "browning" of this canned crab may be due to the dissociation of Cu in crab blood during the heating of the carcasses or during the processing.

3. *Relation between the deterioration of crab blood and the amount of Cu dissociated from haemocyanin*

(1) *Experimental method*

The portions of crude haemocyanin solution having various degrees of decomposition (the amount of V.B.-N were 4.5, 10.8 and 18.9 *mg*%, respectively) were put into collodion bags, and the mouths were sealed.

Each collodion bag was put into a big test tube the mouth of which was

collodion bags heated at the processing temperature

outside of collodion heating at 110°C	Amount of Cu inside of collodion bag after heating		Total amount of Cu in 100 *cc* of sample (%)
Cu outside of bag / Total Cu in sample (%)	in 100 *cc* of sample (%)	Cu inside of bag / Total Cu in sample (%)	
8.9	3.10	63.9	4.85
15.4	2.95	57.8	5.10
—	—	—	—
15.8	2.95	59.8	4.93
11.3	3.65	67.2	5.44
9.1	4.65	73.2	6.35
8.4	5.00	77.4	6.46

plugged with a rubber stopper. Then the test tubes were left at 0°~3°C for 48 hours.

The amounts of Cu in the crude haemocyanin solution in the collodion bag and that which penetrated out from the collodion bag into the big test tube were estimated.

(2) *Results*

Results obtained are shown in Table 28.

As seen in Table 28, with the deterioration of the haemocyanin solution there was increase in the amount of Cu dissociated from the haemocyanin.

Table 28. The amount of Cu dissociated from the crude haemocyanin solution having various degrees of decomposition

V.B-N in the sample (crude haemocyanin solution)	Amount of Cu outside of collodion bag		Amount of Cu inside of collodion bag		Total amount of Cu in the crude haemocyanin (%)
	In 100 *cc* of sample (%)	Cu outside of bag / Total Cu in sample (%)	In 100 *cc* of sample (%)	Cu inside of bag / Total Cu in sample (%)	
4.5	1.32	22.6	4.51	77.4	5.83
10.8	1.68	29.8	3.94	70.2	5.62
18.9	1.92	36.8	3.30	63.2	5.20

Accordingly it may be said that if raw crab carcasses are left for a long time, the molecular construction of haemocyanin in the crab blood will decompose and the large amount of Cu will be freed from the haemocyanin.

X. THE FORMATION OF N-GLYCOSIDES IN BROWNED CANNED CRAB MEAT

It has been stated in the previous papers that the "browning" of canned crab meat may be caused by Maillard reaction, the condensation of sugars (especially, glucose or ribose) and amino acids (such as arginine and histidine)[13,15]. In the procedure of Maillard reaction, it is well known that there are three stages; *i.e.* the initial stage, middle stage and final stage[59,60]. As typical products in each stage, it has been known that N-glycoside appears in the initial stage.

In this article, the formation of N-glycoside will be discussed. Gottschalk and Partridge[60,61,62] have examined the formation of N-glycoside caused by the condensation of amino acids and sugars under a mild condition.

If the "browning" of canned crab is caused by Maillard reaction, there should be N-glycoside which is an initial product in the "browning" reaction in the canned crab meat. Here the present author has tried to separate a product which is considered to be N-glycoside from boiled or canned crab meat; he has examined its properties and found its chemical components by means of paper

chromatography. N-glycoside which was obtained from the model system was compared with that from boiled or browned canned crab meat. The author has thus ascertained the presence of N-glycoside in browned canned crab meat. At last, in order to ascertain the influencing factors upon the proceeding of the initial stage in the "browning" reaction, the factors effecting the formation of N-glycoside were examined in the model system.

A. N-glycoside in boiled crab meat and browned canned crab meat

1. *Sample*

Raw crab meat was separated from the crust and was immediately frozen. After the defrosting of the crab meat in a homogenizer, the meat was crushed. The crushed meat was boiled at 100°C for 20 minutes and was used as a sample. Browned canned crab meat was also crushed and used as a sample.

2. *Separation of N-glycoside*

The separation of N-glycoside was done through the use of boiled crab meat or browned canned crab meat according to a modification of Adachi's[63] method.

A white hygroscopic powder was obtained. The yield was 0.1% from the boiled crab meat and 0.08% from the browned canned crab meat. This white powder is positive to the ninhydrin test or Molisch test. It has a weak reducing power; for example, it slowly discolored methylene blue, and it can not reduce Fehling's solution. The powder becomes brown with heating. The total amount of nitrogen in the white powder from the boiled crab meat was 17.09%, and 16.77% from the browned crab meat. Such properties of the white powder were ascertained to agree with the properties of N-glycoside which were shown by Hodge[59].

The white powder obtained was hydrolyzed with 1N HCl solution, and the amounts of amino nitrogen, and reducing sugar of the hydrolyzate were estimated by the same method as described in a previous article (IV). In order to learn the chemical components of the white powder and its hydrolyzate, an aqueous solution of the white powder and solution of its hydrolyzate were applied to the filter paper (Toyo filter paper No. 50) and chromatographed one-dimensionally by *n*-butanol : acetic acid : water (4 : 1 : 5) for amino acid and sugar. Ninhydrin was used to indicate amino acid and aceton solution of silver nitrate (with alkaline alcohol) for sugar. In the case of the latter reagent, the dried chromatogram was dipped into aceton solution of silver nitrate and dried. Then the paper was sprayed with 0.5 N NaOH in aqueous ethanol. This revealing reagent will be called the alkaline silver nitrate below.

3. *Results*

The total amount of nitrogen in the white powder from the boiled crab meat was 17.09% and 16.77% from the browned canned crab meat. The amount of amino nitrogen in the hydrolyzate of the white powder which was obtained from the boiled crab meat was 2.49%, and the amount of reducing sugar of the same

substance was 2.17%. The ratio of the amount of amino nitrogen to reducing sugar was 1 to 0.87.

The amount of amino nitrogen in the hydrolyzate of the white powder which was obtained from the browned canned crab meat was 2.42% and the amount of reducing sugar of the same substance was 2.07%. The ratio of the two components was 1 to 0.85. The two ratios were almost the same.

The paper chromatograms obtained from the samples which were hydrolyzed or without hydrolysis are shown in Table 29.

Table 29. Paper chromatographic detection of the white powder (separated as N-glycoside) which was separated from boiled crab meat or canned crab meat

Materials from which white powder was separated	Kinds of sample which were chromatographed	Nos. of spots	Revealing reagents	Color of spots	Rf value of revealed spots	Corresponding substance
Boiled crab meat	Aqueous solution of white powder	1	Ninhydrin	Violet	0.08	N-glycoside
		2	Silver nitrate	Pale brown	0.08	
	Hydrolyzed solution of white powder	1	Ninhydrin	Violet	0.10	Arginine
		2	Ninhydrin	Violet	0.12	Histidine
		3	Silver nitrate	Brown	0.14	Glucose
		4	Silver nitrate	Brown	0.27	Ribose
Browned canned crab meat	Aqueous solution of white powder	1	Ninhydrin	Violet	0.09	N-glycoside
		2	Silver nitrate	Pale brown	0.09	
	Hydrolyzed solution of white powder	1	Ninhydrin	Violet	0.10	Arginine
		2	Ninhydrin	Violet	0.12	Histidine
		3	Silver nitrate	Brown	0.14	Glucose
		4	Silver nitrate	Brown	0.27	Ribose

As seen in Table 29, on the paper chromatogram to which was applied an aqueous solution of the white powder obtained from the boiled crab meat or browned canned crab meat, spots of which the Rf values were 0.08~0.09 reacted strongly becoming violet with ninhydrin and reacted weakly becoming pale brown with alkaline silver nitrate. This spot was clarified to be N-glycoside as stated below.

On the other hand, from the hydrolyzate of the white powder two spots respectively revealed by ninhydrin and alkaline silver nitrate were obtained. Rf values of them were 0.10, 0.12, 0.14 and 0.27, respectively. Those Rf values of the spots revealed were compared with those of spots which were obtained from the pure chemical compounds, arginine (Rf 0.10), histidine (Rf 0.12), glucose (Rf 0.14) and ribose (Rf 0.27). The spots on the chromatograms obtained from the hydrolyzate were considered to be due to arginine, histidine, glucose and ribose, respectively. From the results described above, the white powder which was separated from the boiled crab meat or browned canned crab meat seems to be N-glycoside which consists of arginine, histidine, glucose and ribose. Next,

the author undertook to confirm it in a model system.

B. The formation of N-glycoside in a model system

In order to confirm that the white powder obtained from the boiled crab meat or browned canned crab meat is N-glycoside, the author combined arginine, histidine, glucose and ribose which were ascertained to be chemical components of the white powder, for example arginine-glucose, histidine-glucose, arginine-ribose and histidine-ribose systems. Then the properties of N-glycoside which were prepared from the systems described above were investigated, and compared with the properties of the white powder which was obtaind as described in the previous section.

1. *Preparation of N-glycosides*

(1) In the case of the system of arginine-glucose

The condensation of arginine-glucose was carried out after Hamamura and Naito's method[64]. One and decimal *g* of *l*-arginine hydrochloride was suspended in absolute methyl alcohol and an equivalent weight of metallic sodium was added. On the other hand, 0.95 *g* of glucose was added to 200 *cc* of absolute methyl alcohol. The mixture was heated in a water bath with a reflex cooler in order to dissolve the glucose. These two solutions were mixed with the additinon of several drops of glacial acetic acid as a catalyzer. The mixture was further heated for 10 minutes until the mixture became clear. After the evaporation of the methyl alcoholic solution in a vacuum, absolute ethyl alcohol was added to the concentrated solution and white hygroscopic precipitate was obtained. In order to refine it, the white precipitate was dissolved with absolute methyl alcohol, and was filtered. The filtrate was concentrated in a vacuum at low temperature, and absolute ethyl alcohol was added to the concentrated solution. Here white hygroscopic amorphous powder was obtained. The yield was 0.1 *g*.

(2) In the case of the system of histidine-glucose

According to Lewin's method[65], 1 *g* of histidine hydrochloride was suspended in 50 *cc* of absolute methyl alcohol, and an amount of metallic sodium equivalent by weight to the hydrochloric acid in the histidine hydrochloride was added to the methyl alcoholic solution. On the other hand, 1 *g* of glucose was dissolved in 50 *cc* of methyl alcohol. To this methyl alcoholic solution of glucose, the methyl alcoholic solution of histidine was added with three drops of glacial acetic acid as a catalyzer, and the mixture was heated for several minutes in a water bath with a reflex cooler until the mixture solution became clear. After the cooling, the solution was evaporated in a vacuum at low temperature. When ethyl ether was added to the concentrated solution, a mixture of a yellowish white precipitate and yellow mucous substance was obtained. In order to refine the white precipitate, the mixture was dissolved by absolute methyl alcohol and was filtered to remove the mucous substance. When ether was added to the filtrate, a white hygroscopic precipitate was resulted. The yield was 1.4 *g*.

(3) In the case of arginine-ribose

Five-tenths *g* of *l*-arginine hydrochloride and 0.5 *g* of *d*-ribose were employed for condensation the same as in the case of arginine-glucose. Then a yellowish white precipitate was obtained with a yield of 0.18 *g*.

(4) In the case of histidine-ribose

Five-tenths *g* of histidine hydrochloride and 0.5 *g* of *d*-ribose were employed for condensation the same as in the case of histidine-glucose. Then a yellowish brown powder was obtained in a yield of 0.07 *g*.

2. *Comparison of chemical properties of the condensates*

(1) General chemical properties of the condensates

Chemical properties of the four kinds of condensation substances were compared with each other, the properties of the condensation substances in the model systems agreed with those N-glycosides obtained by Hodge[59].

(2) Identification of N-glycosides by paper chromatography

Next, each aqueous solution of N-glycosides obtained above was applied to the paper and chromatographed one-dimensionally by the ascending method in *n*-butanol : acetic acid : water (4 : 1 : 5). The spot revealed by ninhydrin on the paper chromatogram of the condensate of arginine-glucose or histidine-glucose was violet, but the same spot revealed weakly with alkaline silver nitrate was pale brown. The Rf value of the condensates of arginine-glucose or histidine-glucose was 0.07~0.08. The color of the spot revealed by ninhydrin or alkaline silver nitrate of arginine-ribose or histidine-ribose were the same as those from the condensate of arginine-glucose or histidine-glucose. But the Rf value of the condensates of arginine-ribose or histidine-ribose was 0.08~0.09. From the results just noted, it is interesting that the Rf value of the condensates in the model system is not different by the kind of amino acids, but by the kind of sugars. Inoue *et al.*[66] have also offered the same opinion.

In the comparision between the Rf value of N-glycosides obtained from arginine-glucose, histidine-glucose, arginine-ribose and histidine-ribose systems and the Rf values of the condensates (the white powders obtained from browned canned crab meat and boiled crab meat), it was known that each Rf value of N-glycosides obtained in the model systems was contained within the range of the Rf value of the white powder.

(3) Amount of nitrogen-content in N-glycosides

In order to identify the condensate prepared by the combination of amino acids and sugars as N-glycoside, the author has estimated the amount of nitrogen-content in the condensate prepared from arginine and glucose. According to the results obtained, the amount of that content in the condensate was 15.12%. Hamamura[64] has already stated that the amount of nitrogen-content in the arginine-N-glycoside ($C_{13}H_{24}O_7N_4$) is 16.9%. He has considered that in the combination of arginine and glucose, amino radical or ω-position of arginine will combine with glucose because the spot of the condensate was positive in the ninhydrin reaction and was revealed weakly by alkaline silver nitrate in the paper chromatography. His result calculated from his consideration was 16.7%.

The present author's result was almost the same as Hamamura's experimental and calculated values. Thus the properties of the condensate (N-glycoside) prepared by the combination of arginine and glucose have been ascertained. Next, estimation was made of the amount of nitrogen-content in the condensate from arginine and ribose, it was found to be 17.68%. Theoretically calculated amount of nitrogen-content in the compound formed by the combination between arginine and ribose ($C_{11}H_{22}O_6N_4$) was 18.3%. The present author's result was almost the same as the calculated results. Thus the properties of the compound (N-glycoside) formed by the combination of arginine and ribose have been ascertained. In the case of combination of histidine with glucose or ribose, the amounts of nitrogen-content of the two condensates were 14.24% and 15.62% respectively. Lewin's consideration which has been proposed for the combination of amino acid (histidine) with glucose is similar, in part, to the consideration proposed by Katchalsky[67] and Levy[68]. Theoretically calculated amount of nitrogen-content in the compound formed by the combination of histidine and glucose ($C_{12}H_{17}O_7N_3$) was 13.2%, and it was 14.64% in the case of the com-

Table 30. Comparison of the chemical properties between the white substance prepared from boiled or browned canned crab meat and synthesized N-glycosides from arginine or histidine and glucose or ribose systems

Sample / Items	White substance prepared from		Synthesized N-glycoside from	
	Boiled crab meat	Browned canned crab meat	Arginine-glucose Histidine-glucose	Arginine-ribose Histidine-ribose
Reducing power in dilute NaOH soln.				
o-dinitro benzen	Purple after 2 hrs.	Purple after 2 hrs.	Purple after 1 hrs.	Purple after 1 hrs.
Methylen blue	Very slow decoloration	Very slow decoloration	Very slow decoloration	Slow decoloration
Fehling's soln.				
at 25°C	Slight reduction	Slight reduction	Slight or no reduction	Slight or no reduction
at 100°C	Reduction	Reduction	Reduction	Reduction
Molisch's test	Positive	Positive	Positive	Positive
Ninhydrin reaction	Positive	Positive	Positive	Positive
Browning by heating	Slow	Slow	Slow	Slow
Rf value	0.075～0.084	0.076～0.090	0.07～0.08	0.08～0.09
Components obtained by acid hydrolysis	Arginine, Histidine, Glucose and Ribose	Arginine, Histidine, Glucose and Ribose	——	——
Amount of nitrogen-content (%)	17.09	16.77	15.12(Arg.-glucose) 14.24(His.-glucose)	17.68(Arg.-ribose) 15.62(His.-ribose)

bination of histidine and ribose ($C_{11}H_{17}O_6N_3$). Thus the properties of the compounds formed by the combination of histidine-glucose or histidine-ribose have been ascertained respectively.

At last the properties of the white powder of the condensates obtained from boiled crab meat or browned canned crab meat which was considered to be N-glycoside were compared with those of N-glycosides from arginine or histidine and glucose or ribose. The results are compared in Table 30.

As seen in Table 30, the properties of the white substance agreed with the synthesized N-glycosides, so the N-glycosides were considered to be formed in the initial stage of the "browning" reaction of canned crab meat.

Next, factors (pH, heating condition) influencing the formation of N-glycoside in the model system were observed by paper chromatographic method. As results, it was clarified that the formation was the most remarkable in acidic side below pH 5.0, and the formation was completed by heating at 60°C for more than 20 minutes or at 100°C than 10 minutes.

XI. RELATION BETWEEN THE FLUORESCENCE OF RAW OR BOILED CRAB MEAT AND THE "BROWNING" OF CANNED MEAT

According to Patton and Chism[69], after the intermediate stages of Maillard reaction caused by the condensation of amino acids and reducing sugars, fluorescent substances appeared for the first time, if the mixture was exposed under ultra-violet light; absorption peak of the fluorescent substances appeared at 270~280 $m\mu$.

The occurrence of fluorogens is not only observed in the combination of amino acids and glucose or amine-aldehyde, but has also been seen during the storage of many various kinds of food[70,71].

Pearce and Thistle[72] have suggested that it is possible to estimate easily the quality (degree of "browning" of dried eggs) by the estimation of the fluorescence. Nowadays the fluorescence test is being widely employed for the estimation of "browning" of dried eggs, but the characteristics and source of fluorogens are not yet known.

Also in the "browning" of canned crab meat, before the appearance of the brown pigments and the completion of polymerization, the development of fluorescence is assumed to be occurring.

The author has observed the occurrence of fluorescence in browned canned crab meat and investigated the relation between the fluorescence and the "browning" such meat.

A. Relation between the fluorescence in the extractive solution from crab meat and the degree of "browning" of the meat

1. *Preparation of samples*

The whole white crab meat which was prepared as in the previous paper[14] was crushed homogeneously. The aqueous solution extracted from the crushed meat was adjusted to pH 6.8 by the addition of 1 N NaOH, and then crab meat extractive solution without protein was prepared. The amount of amino acid was 170 *mg*% and that of reducing sugar was 192 *mg*%. The solution was heated separately at different condition in order to prepare solutions having various degrees of "browning." The solutions were employed as samples.

2. *Estimation items and methods*

The degrees of fluorescence of the heated extractive were estimated by a fluorescence meter of the A.K.A. photoelectric colorimeter. The wave length of the light was 365 $m\mu$. In the fluorescence test, 0.1 N H_2SO_4 solution of quinon sulfate (0.04 *mg*%) was accepted as 100% of fluorescence. The degrees of "browning" of the heated extractive solutions were estimated by an A.K.A. photoelectric colorimeter as transparency.

3. *Results*

The relation between the degree of "browning" of the heated extractive solutions and the degree of fluorescence is shown in Table 31. The degree of fluorescence of the extractive solutions became higher with increase in degree of "browning". The amounts of amino acid and reducicng sugar in the extractive solutions became smaller with the increase in degrees of "browning" and fluorescence. It is interesting that the extractive solutions heated at the start at 100°C for 20 minutes showed no browning as well as the solutions without heating, nevertheless the heated solutions showed fluorescence.

Table 31. Relation between the degree of "browning" of the heated extractive solutions and the degree of fluorescence

Heating temperature (°C)	Not heated	100°	109°	109°	109°
Items \ Heating time (min.)	0	20	30	60	90
Degree of browning (transparency, %)	99.0	91.5	34.6	12.5	6.0
Fluorescence value (%)	—	3.1	4.3	4.9	5.2
Amount of amino nitrogen (*mg*%)	170	158	157	151	122
Amount of reducing sugar (*mg*%)	192	186	179	136	113

B. Fluorescence in the crab meat during processing

In order to compare the relation between the degrees of "browning" and fluorescence in the extractive solution of crab meat with that of the actual canned crab meat, tests were made of crab meat at various intervals during

processing.

1. *Sample*

The leg meat and shoulder meat of crab were separately employed as samples. At this point, the samples were divided into A-group (having fluorescence under ultra-violet light) and B-group (without fluorescence).

2. *Processing*

The raw crab meat (leg meat and shoulder meat) of A- and B-groups were heated first by "low temperature boiling method" or "high temperature boiling method" and then processed into ¼ pounds cans. During canning, the degrees of fluorescence and "browning" of the crab meat were estimated while it was undergoing the respective processes. In this section of the experiments, the degree of fluorescence was estimated for crab meat and alcoholic extractive solution thereof.

3. *Results*

After opening of the ¼ pounds cans, the degrees of fluorescence and "browning" were measured. The results obtained are shown in Table 32.

Table 32. Relation between the development of fluorescence and the "browning" of crab leg meat at each stage of the process during canning

Groups of raw material		A-group (having fluorescence)		B-group (without fluorescence)	
Sample \ Estimated items		Degrees of browning (reflection ratio, %)	Fluorescence values (%)	Degrees of browning (reflection ratio, %)	Fluorescence values (%)
Raw meat		30.1	3.0	33.7	0
Meat heated by "high temperature boiling method"		50.0	1.2	53.9	0
Meat heated by "low temerature boiling method"		45.2	1.0	44.7	2.0
Canned crab meat prepared from boiled meat	by "high temperature boiling method"	32.5	4.0	43.7	1.0
	by "low temperature boiling method"	30.5	5.0	38.5	2.0

As seen in Table 32, it was clarified that when the solution extracted from the crab meat having fluorescence was heated, the solution became brown, and there was a proportional relation between the degree of the "browning" and the fluorescence value. From those facts, by means of the development of fluorescence in the crab meat heated by the first heating, the likelihood of the appearance of

"browning" in the finished product can be estimated.

In actual practice, if any crab meat subjected to the first heating has fluorescence under ultra-violet light, and if the pieces of meat showing fluorescence are removed from the cans before further processing, finished product of canned crab meat without "browning" will be obtained.

3. A partial characterization of fluorescent substances generated in the "browning" of canned crab meat

As stated in Patron's studies[73], when an aqueous solution of glycine (1 Mol) and glucose (1 Mol) was heated at 100°C for 30 minutes, the resulting solution exhibited strong fluorescence under ultra-violet light although there was only moderate browning in the solution.

Here, the author described his isolation of fluorescent substance from the crab meat and investigation of the chemical characteristics in order to apply the knowledge gained to prevent "browning".

1. *Isolation of fluorescent substance from crab meat*

(1) Sample

The whole white crab meat prepared by the same method as described in the previous paper[14] was left under ultra-violet light, the portions of white crab meat which showed fluorescence were picked out and crushed in a homogenizer. This fluorescent meat was employed as material for these experiments.

(2) Isolation of fluorescent substances

To 200 *g* of the homogenized white crab meat anhydrous sodium sulfate was added to remove water. From the dehydrated white crab meat fluorescent substances were isolated by Lewis and Esselen's method[74] as shown in Scheme 2. Then colorless crystals were formed. When the crystals were separated by centrifuge and dried in a vacuum, plate-shaped colorless crystals as shown in Fig. 10 were obtained. The yield was 0.27%.

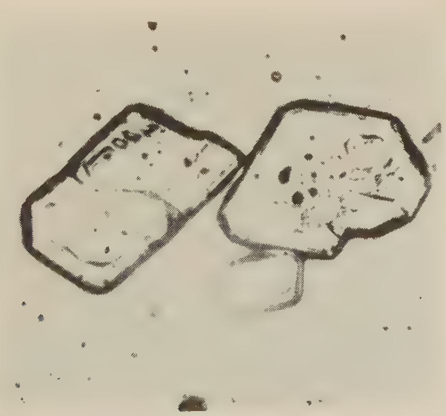


Fig. 10. Plate-shaped colorless crystals having fluorescence under ultra-violet light

Scheme 2. Method of isolation of fluorogens

White crab meat (200 g)
↓
Dehydrate with addition of anhydrous sodium sulfate
↓
Extract with 400 cc of absolute ethanol containing 25 cc of conc. HCl solution
↓
Filter
↓
- Residue
- Filtrate
  ↓
  Concentrate at below 40°C in a vacuum
  ↓
  Separated by centrifuge
  ↓
  - Precipitate (Red substance without fluorescence)
  - Supernatant
    ↓
    Concentrate in a vacuum
    ↓
    Add 1 l of isobutyl alcohol to 200 cc of the concentrated solution
    ↓
    Leave the solution for 24 hours
    ↓
    Separate by centrifuge
    ↓
    - Supernatant
    - Precipitate
      ↓
      Add small amount of butyl alcohol to dissolve the precipitate
      ↓
      Separate by centrifuge
      ↓
      - (Yellowish brown mucous substance)
      - Supernatant
        ↓
        Add ether
        ↓
        Separate by centrifuge
        ↓
        - Supernatant
        - Precipitate
          ↓
          Wash with ether
          ↓
          Dry in desiccator
          ↓
          Plate-shaped colorless crystals (yield, 0.27%)

2. *Characteristics of the fluorescent substance*

The total amount of nitrogen in the isolated fluorescent substance was estimated by Kjeldahl's micro-method to be 3.34 $mg\%$. As the fluorescent substance was positive in ninhydrin reaction and Molisch's reaction, it is considered to possess both an amino group and a carbohydrate group.

A definite amount of the fluorescent substance was hydrolyzed with 1 N

HCl; the amounts of amino nitrogen and reducing sugar in the hydrolyzate were estimated by Van Slyke's method and Somogyi's colorimetric method respectively. The results obtained are shown in Table 33.

Table 33. The amounts of amino nitrogen and reducing sugar in the hydrolyzate of the crystalline substance having fluorescence

Amino nitrogen (%)	1.19
Reducing sugar (%)	2.09

As seen in Table 33, the fluorescent substance was considered to consist of amino nitrogens and reducing sugars at the ratio of 1 to 2. The fluorescent substance was dissolved easily in butyl alcohol and methyl alcohol. It also dissolved in ethyl alcohol, water, dilute hydrochloric acid solution, but did not dissolve in ether.

The fluorescent substance was ascertained to turn brown when it was heated. The relation between the heating temperature and the degree of "browning" of this substance was observed. To a definite amount of the fluorescent substance, water was added to make 0.1% solution containing the substance. Portions of this 0.1% solution were separately heated at 40°~100°C for 10 minutes. After cooling, the degrees of "browning" of the solution and fluorescence were estimated by the same method as described in the previous section. The results obtained are shown in Table 34.

Table 34. Relation between the degree of "browning" when the solution of fluorescent substance was heated and the heating temperature

Heating temperature (°C) / Items estimated	40°	50°	60°	70°	80°	90°	100°
Degrees of browning (transparency, %)	100	99.8	99.5	—	99.5	95.8	95.0
Fluorescence values (%)	2.5	2.5	3.1	—	3.3	3.3	5.2

As seen in Table 34, when the fluorescent substance was heated above 90°C for 10 minutes, it turned brown, but below 90°C for the same period, it did not become brown although the degree of fluorescence increased. Next, investigation was made of whether the degree of "browning" resultant from heating of the fluorescent substance may be influenced by the pH value of the solution or not. The pH values of the solution containing fluorescent substance were adjusted to pH 5.3~8.0 by addition of N/10 NaOH or N/10 HCl solution. Those solutions having various pH values were heated at 100°C for 20 minutes. After

cooling, the degrees of fluorescence and "browning" were estimated by the same method as described in the previous section. The results obtained are shown in Table 35.

Table 35. The influence of pH value on "browning" when the solution of fluorescent substance was heated

pH values of the solution	5.3		8.0	
Items \ Heating	Before heating	After heating	Before heating	After heating
Degrees of browning (transparency, %)	67	57	67	50
Fluorescence values (%)	5.7	8.3	5.7	8.9

As seen in Table 35, the "browning" of the fluorescent substance, when it was heated, was not greatly influenced by the pH value of the solution.

3. *Detection of chemical components in the fluorescent substance*

It was ascertained that the fluorescent substance isolated from crab meat is a chemical substance which will turn brown when heated and that the substance consists of amino acids and carbohydrates. At this point the author made a bromide derivative from the fluorescent substance of which the characteristics were observed, and investigated the chemical components of the fluorescent substance.

(1) Preparation of bromide derivative and its characteristics

The fluorescent substance obtained as described in the previous section 1 was dissolved in ethyl alcohol. To this alcoholic solution a mixture of bromide, potassium chlorate and water (in a ratio 5:1:8) was added until the fluorescence disappeared. This mixed solution was left at 50°C for about one minute and the left at room temperature for 1~2 minutes. Then white plate-shaped crystals of a bromide derivative were obtained as shown in Fig. 11.

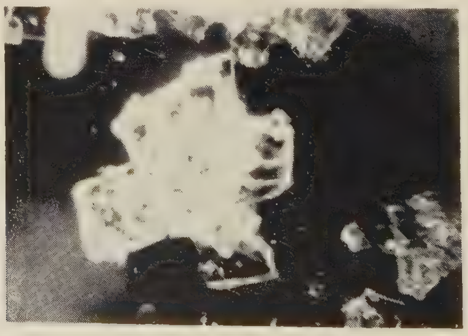


Fig. 11. Crystals of a bromide derivative of the fluorescent substance

This bromide derivative showed positive to Molisch's reaction which fact proves the presence of carbohydrates. This derivative showed negative in Millon's reaction, so there seemed to be not tyrosin or tryptophan content therein. The bromide derivative itself was negative in ninhydrin reaction, but when it was hydrolyzed by 20% HCl solution, the hydrolyzate showed positive ninhydrin reaction. The amount of amino nitrogen in the hydrolyzate was estimated to be 7.1%. The melting point of the bromide derivative was 232°C. The total amount of nitrogen in the bromide derivative was estimated to be 8.42%. The absorption curves of the bromide derivative dissolved in methyl alcohol did not show absorption peak in the range of 250~280 $m\mu$ as shown in curve A in Fig. 12.

From the estimations, the fluorescent substance which is considered to be a precursor of the brown pigment seems to be unsaturated compound which is consisted from amino acids and carbohydrates.

(2) Absorption of the fluorescent substance by magnesium oxide and aluminium oxide column

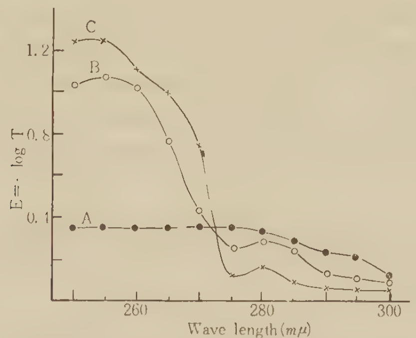


Fig. 12. Absorption curves of the fluorescent substance
Curve A: Bromide derivative of fluorescent substance
Curve B: Fluorescent substance absorbed by magnesium oxide column
Curve C: Fluorescent substance absorbed by aluminium oxide column.

The fluorescent substance obtained as described in the previous section was dissolved in methyl alcohol and then diluted by the addition of 9-fold volume of ether. When the diluted solution was passed through a column (dia. 1.5 *cm*, length 25 *cm*) which was filled with magnesium oxide, the fluorescent substance was absorbed. When methyl alcohol was flowed through the column, bands which gave fluorescence were revealed at the upper surface and at about 3 cm below the surface of the column. As usual the band (lower layer) which was fluorescent was sliced out and extracted with methyl alcohol. The absorption curve of the extracted solution was estimated by photoelectric spectrophotometer of Hitachi type. The absorption curve is shown as curve B in Fig. 12.

The same estimation as in the magnesium oxide column, was carried out in the aluminium oxide column. In this case, the fluorescent band was revealed at about 4~5 *cm* below the upper surface of the column. The band was sliced and extracted with methyl alcohol. The absorption curve is shown as curve C in Fig. 12. As seen in the B and C curves in Fig. 12, the curve of the fluorescent substance absorbed by magnesium oxide showed an absorption peak at 275~285 $m\mu$, and that absorbed by aluminium oxide showed two absorption peaks at 250~255 $m\mu$ and 280 $m\mu$ respectively. Methyl alcoholic solutions of the fluorescent substances absorbed by magnesium oxide or aluminium oxide were employed for the identification by paper chromatography of chemical components of amino acids and carbohydrates. This identification is described in the following section.

(3) Identification of chemical components of amino acids and carbohydrates in the fluorescent substance

In order to identify the chemical components of amino acids and carbohydrates, paper chromatography was used for various samples.

The samples employed were: (a) methyl alcoholic solution of the fluorescent substance which was isolated as described in the previous section, (b) hydrolyzate of the fluorescent substance by 1 N HCl solution, (c) methyl alcoholic solution of the fluorescent substance absorbed by magnesium oxide, (d) methyl alcoholic solution of the fluorescent substance absorbed by aluminium oxide, (e) the hydrolyzate of bromide derivative of the fluorescent substance.

The samples were applied to filter paper and chromatographed one-dimen-

Table 36. Chromatographic identification of sugars and amino acids contained in the fluorescent substance

Nos. of spots	Revealing reagents	Color of the revealed spots: Sample applied to paper chromatogram					Rf values of the revealed spots	Corresponding substance
		a	b	c	d	e		
1	Ninhydrin	—	Pale violet	—	—	Pale violet	0.03~0.04	Unknown
	Silver nitrate	—	Brown	—	Brown	Brown		
2	Ninhydrin	Violet	—	Violet	Violet	Violet	0.07~0.08	N-glycoside
	Silver nitrate	Brown	—	Brown	Pale brown	Brown		
3	Ninhydrin	—	Violet	—	—	—	0.10	Arginine
4	Silver nitrate	—	Brown	—	—	Brown	0.14	Glucose
5	Ninhydrin	Pale violet	Violet	—	—	—	0.12	Histidine
6	Silver nitrate	—	Brown	—	—	Brown	0.26	Ribose

Note: — showed no spot.

sionally by a mixed solution of butyl alcohol : acetic acid : water (4 : 1 : 5) at room temperature for 20 hours. Spots revealed are characterized in Table 36.

As seen in Table 36, the spot of the fluorescent substance or its bromide of which Rf value is 0.07~0.08 corresponds to that from N-glycoside.

The spot (No. 2) disappeared and a few new spots appeared when the fluorescent one or its bromide derivative were developed.

In these facts noted above, the fluorescent substance is considered to be similar to N-glycoside, however, the reducing power of the fluorescent one is more strong than that of N-glycoside and show absorption peak at near 280 $m\mu$ and it turns rapidly to brown in color by heating. From these facts, and Chichester and Mackinney's opinion, the fluorescent substance may be considered to be intermediate substance which is formed in the course of the formation of furfural compound from N-glycoside.

4. *Formation of the fluorescent substance in the model systems*

Friedman and Kline[75] have heated the mixture of various kinds of amino acids with glucose, and observed the relation between the degree of "browning" of the solution and the occurrence of fluorescence, or changes of absorption at the region of ultra-violet.

The present author has heated N-glycoside in order to find out whether the fluorescence will occur or not, and the properties of the fluorogens were compared with those of the fluorogens generated in the crab meat.

(1) Sample

Four kinds of N-glycosides which had been prepared from arginine-glucose, histidine-glucose, arginine-ribose and histidine-ribose were employed in this experiment.

(2) Experimental method

One-tenth % solution of each N-glycoside, of which pH values were adjusted to 6.2 were heated at 60°C for 10 minutes, 100°C for 20 minutes and 110°C for 90 minutes. After the cooling, the pH values, the degrees of the "browning" and then the change of absorption peak were determined.

(3) Experimental results

The results obtained are shown in Table 37. As seen in Table 37, when the solutions were heated at 100°C for 20 minutes, they became only slightly brown in color, but fluorescence occurred. This fact shows that in the middle stage of the "browning" reaction in the system described above, a fluorescent substance was formed.

No absorption peak was observed at the ultra-violet region in the solution of N-glycosides before heating. However, in the solution of N-glycoside which was obtained from amino acids-glucose heated at 100°C for 20 minutes, small absorption peaks at 250 $m\mu$ and 280 $m\mu$ were observed and in that from the amino acids-ribose solution heated at 100°C for 20 minutes, an absorption peak was observed at 280 $m\mu$. The absorption curve changed remarkably with the increase of heating time and rise of heating temperature. The absorption peak

Table 37. Changes of pH, degrees of "browning" and fluorescence values of the heated solutions of N-glycoside at various temperatures

Kinds of N-glycosides obtained from	Items estimated \ Heating condition (°C/min.)	Not heated	60°/10	100°/20	110°/90
Arginine-glucose	pH after heating	6.2	6.0	6.0	6.0
	Degrees of browning (transparency, %)	98.0	97.5	97.0	83.0
	Fluorescence values (%)	0	0	1.0	4.0
Histidine-glucose	pH after heating	6.2	6.2	6.1	6.0
	Degrees of browning (transparency, %)	93.0	92.0	85.0	82.0
	Fluorescence values (%)	0	0	1.0	8.0
Arginine-ribose	pH after heating	6.2	6.2	6.1	6.0
	Degrees of browning (transparency, %)	99.1	98.0	97.0	85.5
	Fluorescence values (%)	0	0	2.0	5.9
Histidine-ribose	pH after heating	6.2	6.2	6.1	6.0
	Degrees of browning (transparency, %)	99.0	98.0	96.5	83.2
	Fluorescence values (%)	0	0	2.1	6.7

of the solution of N-glycosides which were obtained from glucose and amino acids is situated in shorter wave length side than that of ribose and amino acids. Fluorescent solution of N-glycosides (obtained in arginine-glucose system or arginine-ribose system) which were prepared by heating at 110°C for 90 minutes were separately passed through a magnesium oxide column as treated in the previous section. Next, about 50 *cc* of methyl alcohol was run through each column. Then, there appeared a fluorescent band at several *cm* below the surface of the column. The layer of the fluorescent band in each column was sliced out and extracted with methyl alcohol. The absorption curve of the extract was obtained. According to the results obtained, the fluorescent substance which was prepared from the heated glycoside of arginine-glucose showed the absorption peak at 255~265 $m\mu$ and the one which was prepared from the glycoside of arginine-ribose showed the peak at 280~284 $m\mu$. The extract became brown with heating, and was positive by Molisch test.

The paper chromatograms of the extract and its hydrolyzate were obtained. Paper chromatograms of the fluorescent substances obtained from arginine-glucose system or arginine-ribose system showed one spot each revealed by alkaline silver nitrate or ninhydrin. The Rf value of the spot obtained from the

former system was 0.07~0.08 and that of the latter system was 0.08~0.09. Paper chromatograms from each hydrolyzate of the fluorescent substance showed two spots revealed by silver nitrate. The spot of which the Rf value was 0.13~0.14 was that of glucose and the spot of which the Rf value was 0.26~0.27 was that of ribose. The spot revealed by ninhydrin of which the Rf value was 0.10 was arginine.

XII. THE FORMATION OF HYDROXYMETHYLFURFURAL IN "BROWNING" OF CANNED CRAB MEAT

In the process of the Maillard reaction, it has been found that N-glycosides, some fluorescent substance, reducton and hydroxymethylfurfural are present as intermediate substances. It is important to ascertain the process of the formation of hydroxymethylfurfural in order to know the mechanism of "browning" of canned crab meat. Patton and Josephson[76] have isolated furfural alcohol, furfural-5-hydroxymethylfurfural (H.M.F.)[77], maltol[78], and acetol[79] as intermediate substances in the "browning" of heated milk. Stadtman[80] and Täufel[81] have studied the "browning" reaction in a glucose-glycine system and have detected the formed substance by paper chromatography and have ascertained the presence of hydroxymethylfurfural. From these points the present author has detected the presence of hydroxymethylfurfural in canned crab meat by paper chromatography, absorption spectrophotography and polarography.

A. Detection of hydroxymethylfurfural from browned canned crab meat

1. *Experimental method*

Normal canned crab and browned canned crab meat were employed for the sample. Two hundred *g* of each sample canned crab meat was crushed in a homogenizer and extracted with ether. The extract was warmed and ether was removed. The residue was dissolved by the addition of 20 *cc* of distilled water and used as a sample for the estimation. The absorption curve of the solution was determined by a photoelectric spectrophotometer. A part of the solution was applied to filter paper (Toyo filter paper No. 50) and chromatographed one-dimension by a developing reagent of *n*-butanol : acetic acid : water (4 : 1 : 5). For the revealing reagent, alkaline silver nitrate solution was used as previously. Last, to 2 *cc* of the sample solution 0.1 N NH_4Cl solution was added to make the total volume 10 *cc*; this solution was employed to detect the reduction wave of H.M.F. in a polarogram.

2. *Experimental results*

(1) Absorption curves of the solution extracted from normal or browned canned crab meat

The absorption curves of the extracts of normal or browned canned crab meat are shown in Fig. 13.

As seen in Fig. 13, the extract from the browned canned crab meat showed

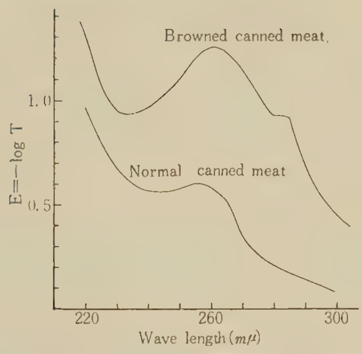


Fig. 13. Absorption curves of the extracts of normal or browned canned crab meat

absorption peaks at 264 mμ and 284 mμ. On the other hand, the extract from the normal canned crab meat showed the absorption peak at 259 mμ. The chemical components showing absorption peaks at 264 and 284 mμ were considered to be levulinic acid and H.M.F. respectively, because Nomura and Kono[82,83] have detected levulinic acid and H.M.F. at the same absorption peaks respectively.

(2) Paper chromatography

The paper chromatograms obtained from the samples prepared from normal canned crab and browned canned crab meat are shown in Table 38.

Table 38. Paper chromatographic detection of hydroxymethylfurfural in normal and browned canned crab meat

Nos. of spots	Revealing reagents	Rf values of revealed spots	Color of spots		Corresponding substance
			Normal canned crab	Browned canned crab	
1	Silver nitrate	0.76	Brown	—	Reducton[83]
2	Silver nitrate	0.85	—	Brown	H. M. F.[60][80]
3	Silver nitrate	0.95	—	Brown	Unknown

As seen in Table 38, two spots of which the Rf values were 0.85 and 0.95 respectively were revealed by an alkaline silver nitrate in the extract of browned canned crab meat. On the other hand, only one spot revealed by the same reagent was obtaind from the extract of normal canned crab meat. The spot of which the Rf value is 0.85 was clarified to be H.M.F. by Gottschalk[61] and Chichester, Stadtman and Mackinney[81]. The spot of which the Rf value is 0.76 was assumed to be reducton from Ikeda's results[85].

(3) Polarography

The polarograms of the extracts of normal or browned canned crab meat are shown in Fig. 14.

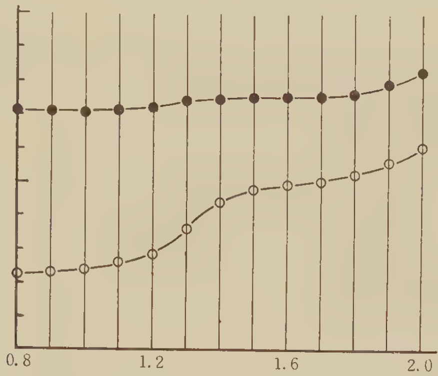


Fig. 14. Polarograms of H.M.F. in normal or browned canned crab meat
●……Normal canned meat ○……Browned canned meat.

As seen in Fig. 14, the polarographic wave from the extract of the browned canned crab meat showed two steps at −1.35 and −1.81 of half wave potential ($E_{1/2 \cdot h}$). On the other hand, the polarographic wave from the extract of the normal sample showed one step at −1.91 of $E_{1/2 \cdot h}$ value. The step at −1.35 of $E_{1/2 \cdot h}$ is considered to show the presence of H.M. F. Nomura[83] by polarography has detected H.M.F. showing $E_{1/2 \cdot h}$ value of −1.35, in browned orange juice. Another step showing −1.91 of $E_{1/2 \cdot h}$ value may be acetaldehyde[86], but it is not ascertained.

From the results obtained by the photoelectric spectrophotometer, paper chromatography and polarography, the presences of H.M.F. and its derivative (levulinic acid) as intermediate products were ascertained.

B. Formation of hydroxymethlylfurfural in a model system

1. *Experimental method*

One-fifth solutions of glucose, ribose and *l*-arginine each were employed for samples, and 1/5 Mol solution of glucose and 1/5 Mol solution of *l*-arginine or 1/5 Mol. solution of ribose and 1/5 Mol solution of *l*-arginine were respectively mixed with each other in equal volumes. Each mixture was heated at 100°C for 20 minutes or 110°C for 90 minutes. After cooling the solutions were used for the measurement of the absorption curve by a photoelectric spectrophotometer. Other parts of each heated solution were applied to filter paper and chromatographed by the same method as described in the previous section.

At last each 2 *cc* of the heated solutions was neutralized with NaOH solution, and 0.1 N NH_4Cl was added to the neutralized solution making the total volume

up to 10 *cc*. The resulting solutions were used as sample solutions for obtaining a polarogram with Shimadzu's apparatus, the reduction wave of H.M.F. was determined.

2. *Experimental results*

(1) Absorption curves of the heated solution of arginine-sugars

From the absorption curves, the absorption peak at 284 $m\mu$ was found in the solution of arginine-glucose or arginine-ribose which was heated at 100°C for 20 minutes. The absorption peak from the heated solution of arginine-glucose at 110°C for 90 minutes was seen at 264 $m\mu$.

According to Nomura[83,86], Chichester[84] and Wolfrom[87] the absorption peak of H.M.F. was seen at 285 $m\mu$, and that of levulinic acid which was changed from hexose *via* H.M.F. was seen at 264 $m\mu$. From these results, in the absorption curves from the solution of arginine-glucose, the chemical components on account of which the absorption peak was seen at 284 $m\mu$ was considered to be H.M.F.

Table 39. Paper chromatographic detection of chemical components formed in the mixed solutions of arginine-sugars by heating

Systems	Conditions of heating	Nos. of spots	Revealing reagents	Rf value of spots	Corresponding substance
Agrinine-glucose system	100°C 20 min.	1	Ninhydrin Silver nitrate	0.07~0.08	N-glycoside
		2	Ninhybrin	0.10	Arginine
		3	Silver nitrate	0.13~0.14	Glucose
	110°C 90 min.	1	Ninhydrin Silver nitrate	0.07~0.08	N-glycoside
		2	Silver nitrate	0.05~0.06	Unknown
		3	Silver nitrate	0.13~0.14	Glucose
		4	Silver nitrate	0.60	Unknown
Arginine-ribose system	100°C 20 min.	1	Ninhydrin Silver nitrate	0.08~0.09	N-glycoside
		2	Silver nitrate	0.26~0.27	Ribose
		3	Ninhydrin	0.10	Arginine
	110°C 90 min.	1	Ninhydrin Silver nitrate	0.08~0.09	N-glycoside
		2	Silver nitrate	0.03	Unknown
		3	Silver nitrate	0.05~0.06	Unknown
		4	Silver nitrate	0.26~0.27	Ribose
		5	Silver nitrate	0.84~0.85	H. M. F.[60)80)]

and that at 264 $m\mu$ was considered to be levulinic acid which was formed from hexose *via* H.M.F.

(2) Paper chromatography

Paper chromatograms from the heated solution of arginine-sugars are shown in Table 39.

As seen in Table 39, on the paper chromatogram of the aqueous solution heated at 110°C for 90 minutes, two spots revealed by silver nitrate appeared at the position of Rf values 0.05~0.06 and 0.60 in addition to the three spots: glucose, arginine and N-glycoside. The spot showed also on the paper chromatogram of the hydrolyzed solution of glucose, but the spot was not given by the hydrolyzed solution of ariginine. Thus the source of the spot was considered to be a hydrolyzed product of glucose; however, the chemical compounds of the spot were not identified. The spot of which the Rf value is 0.60 was suggested to be reduction in contrast with the conclusions reached by Euler and Martius[88], Adachi[63] and Ikeda[85].

On the paper chromatograms from the solution of arginine-ribose heated at 110°C, three spots additional to the spots of ribose and N-glycosides were newly revealed. The spots of which the Rf values were 0.03 and 0.05~0.06 were shown also on the paper chromatogram from the hydrolyzed solution of ribose, but were not given from the hydrolyzed solution of arginine. Thus the source of the spots were considered to be a sugar derivative, but the chemical components were not identified. A spot having a Rf value of 0.84~0.85 which was revealed by silver nitrate was clarified to be H.M.F. from the experimental results of Gottschalk[62], Chichester, Stadtman, Mackinney[84], and Nomura[86].

(3) Polarograms from the heated solution of arginine-sugars

On the polarogram from the solution of arginine-ribose heated at 100°C for 20 minutes, there was no step of half wave potential ($E_{1/2 \cdot h}$). However, when the solution was heated at 110°C for 90 minutes, the polarographic wave from the solution showed one step at −1.35 of a half wave potential ($E_{1/2 \cdot h}$). This value of $E_{1/2 \cdot h}$ corresponding to that of H.M.F. as an intermediate product in this "browning" reaction was ascertained in the "browning" of the model system as well as in the "browning" of canned crab meat.

XIII. BROWN PIGMENT SUBSTANCES IN THE BROWNED CANNED CRAB MEAT

It is known that the end product, a brown pigment, in the Maillard reaction of foods is melanoidins[89]. For example, Weast and Mackinney[25] has isolated melanoidins from browned dried apricots, and has ascertained that the chemical components which take part in the "browning" reaction of dried apricots were aspartic acid and glutamic acid as amino acids and fructose as carbohydrate.

The present author has suggested that the "browning" reaction of canned crab meat also takes place as a result of the polimerization of amino acids (arginine or histidine) and sugars (glucose or ribose)[14]. As to this point, in order to check this suggestion, the author has isolated the brown pigment substances and investigated their characteristics and chemical components.

A. Isolation of brown pigment substance from browned canned crab meat

1. *Sample*

Browned canned crab meat which had failed to pass the export inspection was employed as the material. The red epidermis of the browned canned crab leg and shoulder meats was removed and the brownish white meat was crushed by means of a homogenizer. The crushed meat was dried at a low temperature in a vacuum. Thus about 40 *g* of browned crab meat was obtained.

2. *Method of isolating browned pigments*

The dried browned crab meat was treated according to Weast's method[25] as shown in Scheme 3, and brown pigment was isolated.

3. *Partial characteristics of the isolated brown substances*

In Scheme 3, Part I-1 was reddish brown, II-1 was blackish brown and III-1 was yellowish brown. Those pigment substances which were insoluble with ether exhibited fluorescence under ultra-violet light. But the pigment substances in parts I-2, II-2 and III-2 which were soluble in ether had no fluorescence.

Each brown substance which was extracted by means of various solvents reduced Fehling's reagent and reacted positively with ninhydrin. Also, these brown substances were easily dissolved in alkaline solution, but were dissolved with difficulty in water. An alkaline solution of each substance was applied to filter paper (Toyo filter paper No. 50) and was chromatographed by the one-dimensional ascending method.

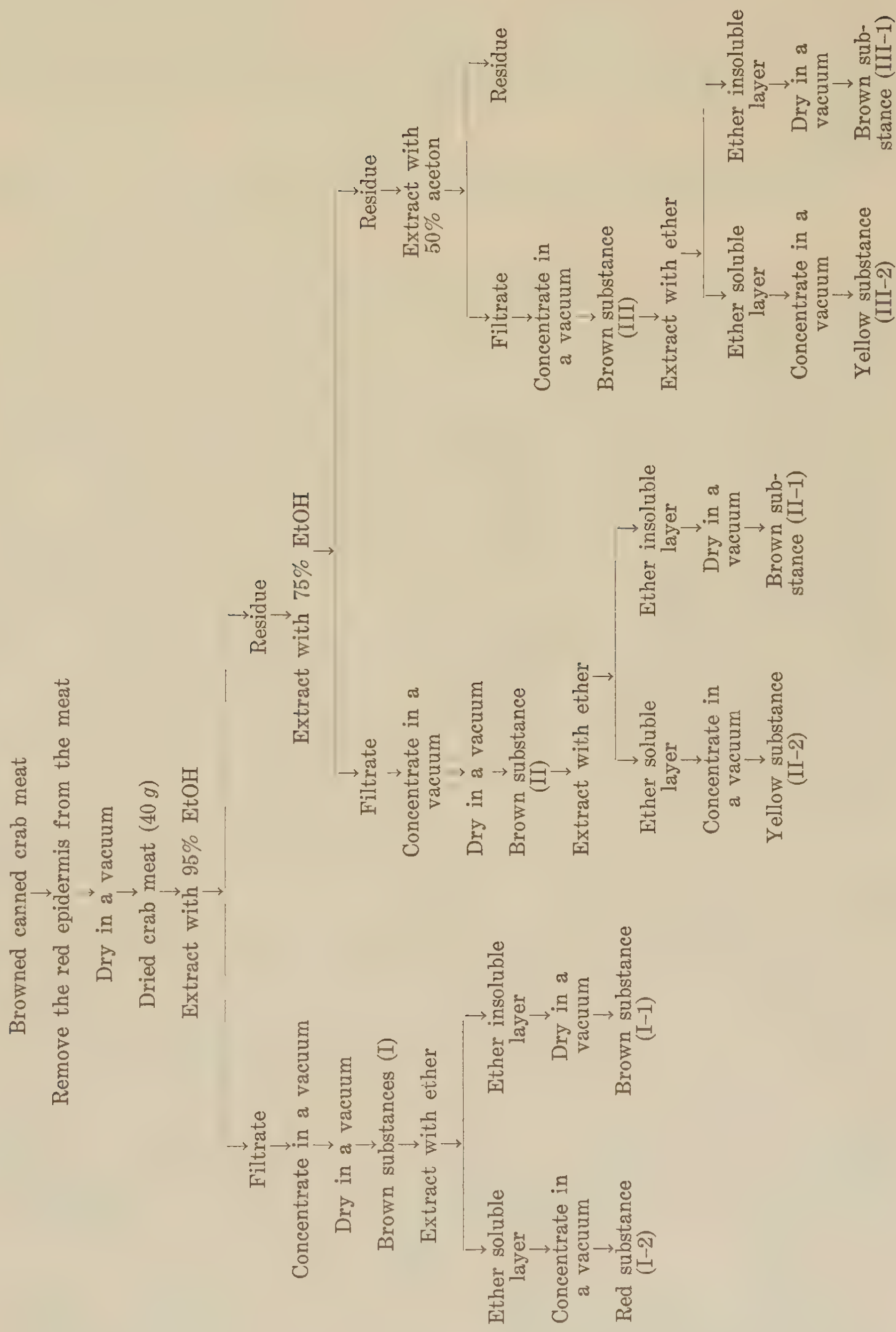
From the result of chromatographic detection, in each brown substance the certain chemical compounds present reacted with ninhydrin or silver nitrate. Among the chemical compounds, arginine, histidine, glucose, ribose and N-glycoside were ascertained in contrast with chromatograms of pure chemical compounds.

B. Fractionation of the isolated brown pigment substance from brown canned crab meat

The author, next, undertook to refine the isolated brown pigment substances and to investigate their chemical characteristics.

1. *Refining*

Samples I-1, II-1, III-1 which were obtained as described in the previous section were dissolved by means of N/10 NaOH solution, respectively. Each solution was acidified by addition of 1 N HCl, each solution was shaken, then the mucous brown substance precipitated. The precipitates were dialyzed in a collodion bag against distilled water for five days. The mucosity of the brown substances decreased. The brown substances were dissolved with N/50 NaOH



Scheme 3. Isolation of brown pigment by Weast's method

and then 1 N HCl was added to the solution to acidify, then the brown substances precipitated. After the precipitate was filtered, the brown substances were dialyzed by means of the same treatment as the above. After two times dialysis, the brown susbtances were dried in a vacuum. Finally samples A, B and C of brown powder substances were obtained from samples I-1, II-1 and III-1, respectively.

2. *Some characteristics of the refined brown substances*

Some characteristics of the refined brown substances are shown in Table 40.

Table 40. Some characteristics of the refined brown substances

Kinds of the brown substances / Characteristics	A	B	C
Appearance of the substances	Brownish red mucus	Brownish black powder	Yellowish brown powder
Protein test	Negative	Negative	Negative
Ninhydrin test	Slightly positive	Negative	Negative
Reducing power to Fehling's solution at 25°C at 100°C	Negative Positive	Negative Positive	Negative Positive
Changes by the addition of bromine	Discolored reversibly	Discolored reversibly	Discolored reversibly
Changes by the addition of sodium hypochlorite	Discolored irreversibly	Discolored irreversibly	Discolored irreversibly

Those refined brown substances showed strong fluorescence under ultra-violet light and were easily soluble in an alkaline solution, but were dissolved with difficulty by an acidic solution. The easy solubility by an alkaline solution agreed with the practical experience that if the browned canned crab meat was heated with an alkaline solution above pH 8.0, the brown pigment dissolved into the solution and the meat became white. Those refined brown substances were discolored easily by sodium hypochloride solution, and became white as a result of heating. This change was irreversible. If those refined brown substances were treated with bromine, they were slightly discolored to a pale orange. This change was reversible. So if the pale orange substances were heated to remove bromine, they turned brown again. Those characteristics of samples A, B and C resembled those of melanoidins[90,91], which have been said to be end-products of the amino-carbonyl reaction.

C. Detection of chemical components of the refined brown pigment substances derived from brown canned crab meat

Carson and Olcott[92] have made it clear that melanoidins as an end product

in the acetaldehyde-amine system were a condensation product which were formed by the dehydration of two molecules of water from one molecule of amine and four molecules of acetaldehyde. It is important to detect the chemical components in the brown pigment of the browned canned crab meat in order to ascertain whether or not the pigment agrees with some melanoidin which has been studied by many investigators[25,92-94].

The present author has hydrolyzed the refined brown pigment and detected the chemical components of the hydrolyzate by paper chromatography.

1. *Samples and procedure*

Employed in this experiment were the brown pigment substances, samples A, B and C which were isolated from the browned crab meat and were refined as described in the previous section. The total amount of nitrogen of the samples was estimated by Kjeldahl's micro-method. A definite amount of each sample was poured into separate small test tubes with 1 N HCl solution; the test tubes were sealed by fusion. The sealed test tubes were heated in boiling water for 12 hours. After the hydrolysis, the hydrolyzate was neutralized with 1 N NaOH solution and filtered. Each definite volume of this solution was employed for the estimations of the amount of amino nitrogen by Van Slyke's method and reducing sugar by Somogyi's method, respectively. On the other hand, in order to know the chemical components of the brown pigments and their hydrolyzates, 1 N NaOH solutions of brown pigments A, B and C together with the hydrolyzates were paper chromatographed by the one-dimensional ascending method with the mixture of *n*-butanol : acetic acid : water (4 : 1 : 5). The revealing reagents were the same as those described in the previous section. Absorption spectra of dilute alkaline (N/200 NaOH) solutions of the brown substances A, B and C which were obtained from the browned canned crab meat were estimated by Hitachi photoelectric spectrophotometer.

2. *Results*

(1) Total amount of nitrogen, amount of amino nitrogen and reducing sugar in the brown substances A, B and C

Results of the analysis are shown in Table 41.

Table 41. Results of analysis of the refined brown pigment substance and its hydrolyzate

Items \ Kinds of sample	A	B	C
Total-N in the brown pigment (%)	4.625	5.970	2.660
Amino-N in the hydrolyzate of brown pigment (%)	0.222	0.569	0.151
Reducing sugar in the hydrolyzate of brown pigment (%)	0.233	0.299	0.123

As seen in Table 41, the ratio of amino nitrogen to reducing sugar was 1 to 1 in A, 2 to 1 in B and 1 to 1 in C.

(2) Paper chromatography

The amino acid and sugar components in the 1 N NaOH solution of the refined brown pigment substances and their hydrolyzates, which were detected by paper chromatography, are shown in Table 42.

Table 42. Paper chromatographic detection of constituents of the brown substances A, B and C obtained from the browned canned crab meat

Kinds of sample employed	Nos. of spots	Revealing reagents	Rf values of revealed spots	Color of the revealed spots			Corresponding substances
				A	B	C	
1 N NaOH solution of the brown pigments	1	Silver nitrate	0	Brown	Brown	Brown	Melanoidins
	2	Ninhydrin	0.08	Violet	—	—	Unknown
Hydrolyzates of the brown pigments	1	Silver nitrate	0.03	Pale brown	Pale brown	Pale brown	Unknown
	2	Silver nitrate	0.05	—	—	Brown	Unknown
	3	Ninhydrin Silver nitrate	0.07 0.07	Violet Brown	Violet Brown	Violet —	N-glycoside
	4	Ninhydrin Silver nitrate	0.09 0.09	— —	— —	Violet Pale brown	N-glycoside
	5	Silver nitrate	0.13	Brown	Brown	Brown	Glucose
	6	Silver nitrate	0.27	Brown	Brown	Pale brown	Ribose
	7	Silver brown	0.7	Pale brown	Pale Brown	—	Reducton[85]
	8	Silver nitrate	0.85	—	Brown	—	H. M. F.[83]

As seen in Table 42, in the chromatogram obtained from 1 N NaOH solution of each refined brown pigment substance such as samples A, B and C, only one spot each which did not move from the origin was revealed by spraying of silver nitrate respectively, but from the hydrolyzates of samples, many spots were revealed, respectively.

(3) Absorption spectrum in the ultra-violet region

The obtained absorption curves are shown in Fig. 15.

As seen in Fig. 15, in case of the brown substances A and B, two peaks

developed in the ultra-violet absorption spectra at 278~280 and 290 $m\mu$. On the other hand, a peak developed in the ultra-violet spectrum at 280 $m\mu$ in the brown substance C.

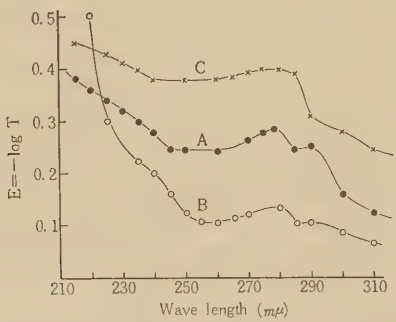


Fig. 15. Absorption curves of the brown substances A, B and C from browned canned crab meat

D. Formation of brown substance in the model system

It has been known that in the final stage of "browning" caused by Maillard reaction, the brown pigment is formed. As described in the previous section, the present author has made it clear that the brown pigment separated from the browned canned crab meat consists of arginine and histidine as amino acids and glucose and ribose as sugars, at least. At this point, observations were made of the chemical properties of the brown pigment formed from model systems of arginine-glucose or arginine-ribose, and comparison was made with the pigment from browned canned crab meat.

Sattler and Zerban[95] have separated brown pigment, melanoidin, from dried apricots and have ascertained that the melanoidin consists of aspartic acid and fructose, that it has no hexose, that it is negative to anthron test and that it has no hydroxymethylfurfural. But when they hydrolyzed the melanoidin which was condensated at 100°C from aspartic acid and fructose which are the chemical components of the melanoidins, they observed the presence of hexose and hydroxymethylfurfural.

1. *Formation of melanoidin from arginine-glucose system and its properties*

After Enders' standard method[90,93] for the preparation of "standard melanoidin", the present author has tried to prepare melanoidin from arginine-glucose system. The brown pigment substances, I, II and III were obtained. Brown pigment substance I was yellowish brown, II was reddish brown and III was dark brown. Those pigment substances showed fluorescence under ultraviolet light. Then the brown substances, I, II and III were extracted with ether in order to remove hydroxymethylfurfural or reducton. Thus, the brown substance was fractionated into two parts, an ether-soluble part and an ether-

insoluble part. Each part was concentrated in a vacuum at low temperature. The pigment substances which were obtained from the ether-soluble part were called I-2, II-2, and III-2; they were yellowish brown in color and showed no fluorescence under ultra-violet light. On the other hand, the pigment substances obtained from the ether-insoluble fraction were called I-1, II-1 and III-1. The pigment substance I-1 was yellowish brown in color, II-1 was dark brown and III-1 was reddish brown. Those pigment substances showed fluorescence when they were left under ultra-violet light. The brown pigment substances I-1, II-1 and III-1 were washed with methyl alcohol in order to remove free arginine, glucose and N-glycoside by which the substances were contaminated. Resulting pigments were washed with ether several times and were dried under reduced pressure. Then brown powders, A, B and C were obtaind from the pigment substances I-1, II-1 and III-1 respectively.

2. *Chemical properties of the brown pigment*

(1) General chemical properties

The brown pigment substances which were refined as described in the previous section were slightly soluble in water, especially in alkaline water. The chemical properties of the brown pigment substances were almost the same as the brown substances which were isolated from browned canned crab meat.

(2) Amounts of amino nitrogen and reducing sugar in the hydrolyzates of the brown substances A, B and C

Total amount of nitrogen of the brown substances A, B and C which were refined as related in the previous section were estimated by Kjeldahl's micro-method. Also, in order to ascertain the ratio of amino acid and sugar constituting the brown substances A, B and C, the amount of amino nitrogen and the amount of reducing sugar in the hydrolyzates of the materials were estimated by Van Slyke's method or Somogyi's method respectively. The results estimated are shown in Table 43.

Table 43. Amounts of amino nitrogen and sugar in the hydrolyzates of the brown substances, A, B and C

Kinds of brown substances / Items estimated	A	B	C
Amount of amino nitrogen (%)	0.190	0.136	0.158
Amount of reducing sugar (%)	0.169	0.062	0.128

As seen in Table 43, the ratio of amino nitrogen to reducing sugar was 1 to 1 in A, 2 to 1 in B and 1 to 1 in C. Those ratio were the same as those of the brown substances A, B and C which were fractionated from browned canned crab meat as described in the previous section.

(3) Paper chromatography

Alkaline solutions of the brown substances, A, B and C were applied to filter paper and chromatographed by the one-dimensional ascending method with the same developing reagent as those used in the procedures described in the previous section. The revealing reagent was the same as in the previous section.

On the paper chromatogram, from each of the brown substances A, B and C, two spots which are revealed strongly by alkaline silver nitrate at the Rf values of 0 and 0.028~0.03, were given. The spot which did not move from the origin was considered to correspond with a spot of melanoidin in view of the result obtained by Carson[92]. On the other hand, the compound corresponding to the spot of which the Rf value is 0.028~0.03 is unknown.

(4) Absorption spectrum in the ultra-violet region

The absorption spectra in the ultra-violet region of the dilute alkaline solution (N/200 NaOH) of the brown substances A, B and C were studied by means of the same apparatus as used in the previous section.

From the obtained curves, the absorption peak appeared at 278~280 $m\mu$ in brown substance A, two peaks at 285 $m\mu$ and 278 $m\mu$ appeared in substance B, and one peak at 282 $m\mu$ appeared in substance C. Those absorption peaks agreed with those in the brown substances obtained from browned canned crab meat.

3. *Identification of the brown substances which were separated from the browned canned crab meat or from the model system*

According to Maillard's definition[22,90], the dark colored substances formed by heating amino acids and sugars together are called melanoidins. The colored substances containing nitrogeneous compounds show fluorescence under ultra-violet light, do not reduce Fehling's solution and react reversibly with bromine. However, the properties of the substances vary according to the methods of preparation and it is probable that none of the melanoidin prepared by many investigators is all composed of a single compound. Methods for the preparation and purification have been given by Enders[93], Enders and Theis[96]. The properties of the brown substances which were prepared by a modification of Enders' method[93] from the condensation of arginine-glucose, agreed with the properties of standard melanodin.

In this section, in order to make certain of the suggestion that the "browning" reaction of canned crab meat is caused by Maillard reaction between amino acids and sugars, the present author has compared the properties of the brown substances separated from browned canned crab with those of the brown substances prepared from the condensation of amino acid and sugar through the standard method[93] for the preparation of melanoidins. Comparison of properties of these brown substances is shown in Table 44. As seen in Table 44, the properties of the brown substances separated from browned canned crab meat agreed with those of the substance prepared by the condensation of arginine and glucose in a model case. From these results, it was ascertained that the "browning" of canned crab meat is caused by the Maillard reaction between amino acids and sugars.

Table 44. Comparison of properties of the brown substances, A, B and C, separated respectively from the browned canned crab meat or from the condensation of amino acid and sugar

Brown substances fractionated by		95 % EtOH (A)		75 % EtOH (B)		50 % Aceton (C)	
Items estimated / Brown substances obtained from		Browned canned crab	Model system	Browned canned crab	Model system	Browned canned crab	Model system
Appearance		Brownish red mucus	Yellowish brown powder	Brownish black powder	Brownish black powder	Yellowish brown powder	Brownish red powder
Protein test		Negative	Negative	Negative	Negative	Negative	Negative
Ninhydrin test		Slightly positive	Negative	Negative	Slightly positive	Negative	Slightly positive
Reducing power to Fehling's solution	at 25°C	Negative	Negative	Negative	Slightly positive	Negative	Slightly positive
	at 100°C	Positive	—	Positive	—	Positive	—
Change of color by the addition of	Bromine	Discolored reversibly	Discolored reversibly	Discolored reversibly	Discolored reversibly	Discolored reversibly	Discolored reversibly
	Sodium hypochlorite	Discolored irreversibly	Discolored irreversibly	Discolored irreversibly	Discolored irreversibly	Discolored irreversibly	Discolored irreversibly
Solubility of brown substance for various solvents	Acidic water	Insoluble	Slightly soluble	Insoluble	Slightly soluble	Insoluble	Slightly soluble
	Alkaline water	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
	Organic solvents	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Total-N in brown substance (%)		4.63	5.02	5.97	2.94	2.66	3.47
Amino-N in the hydrolyzate (%)		0.222	0.190	0.569	0.136	0.151	0.158
Reducing sugar in the hydrolyzate (%)		0.233	0.169	0.299	0.06	0.123	0.128
Ratio of amino-N to reducing sugar		0.96 : 1	1.1 : 1	1.9 : 1	2.2 : 1	1.2 : 1	1.2 : 1
Absorption peaks appeared at ($m\mu$)		270~280, 290	278~280	278~280, 290	278, 285	280	282

XIV. GENERAL DISCUSSION

The author has studied the phenomena of the "browning" of canned carb meat, and has discussed the factors influencing the occurrence of the "browning" in the previous articles (II, IV, V, VII, VIII, IX). Now the author will state his conclusions as to the cause of the "browning", and explain its mechanism and the methods for prevention.

A. The reasons why the Maillard reaction has been considered as the cause of the "browning" of canned crab meat

1. *Preliminary consideration*

"Browning" is observed frequently during the processing and preservation of certain foods. The causes of the "browning" have been considered to be: (1) the Maillard reaction, (2) caramelization, (3) the oxidation of fat and oil, and (4) enzymatic oxidation. In canned marine foods, another type of discoloration has often been observed, which is the blackening of the contents caused by the combination of the metal of the can container and hydrogen sulfide generated from the contents.

In the beginning of studies on the "browning" of canned crab meat, the reaction named above was considered as having causal significance. However, the "browning" reaction resultant from enzymatic action or oxidation of fat seemed to be not among causes of the "browning" of the canned crab meat, because canned crab containers, in which the ungreasy boiled meat is packed, are sealed under a vacuum for sterilizing at a relatively high temperature (6 pounds pressure, 110°C). As characteristic properties of canned crab meat, it is known that its fat content is 0.29~0.45%[97,98] which amount is smaller in comparison with that, generally 2.5%, in canned fish meat[1]. Also enzymatic action in crab meat is inactivated by heating the meat above 80°C for 10 minutes[51].

On the other hand, the combination between hydrogen sulfide generated from the crab meat with Cu contained in crab blood may be taken into consideration, but the combined product is blue. So the combination is not a cause of the "browning".

Another type of "browning" called caramelization was considered. In the course of caramelization, similar brown substances are formed as in the amino-carbonyl reaction, but the reaction occurs when certain foods having a great amount of carbohydrates are heated to a high temperature in the absence of amino compounds[59,90]. In respect to this point, the "browning" of canned crab meat may rarely be caused by this reaction just noted, because a large amount of amino compounds are contained together with sugars in canned crab meat (Article III)[98].

From the considerations described above, the cause of the "browning" of

canned crab meat comes down to the Maillard reaction due to the combination of amino compounds and sugars.

It has been clarified that the Maillard reaction is the condensation reaction between the amino radical of amino acids and the aldehyde radical of sugars; the generation of a caramel odour[99], occurrence of fluorescence, appearance of an absorption peak in ultra-violet regions and decreases of amino acids and sugars accompany the reaction as it continues.

In respect to the points noted above, the present author at first deduced that the "browning" would be caused to take place by the Maillard reaction. The above described studies on the "browning" of canned crab meat were carried out with a view to verifying this assumption. Comparison has been made of the chemical components and other characteristics of the normal canned crab meat with those of the browned canned crab meat. From the results obtained[13], the following differences may be listed:

1) The amounts of free amino acids and sugars in browned canned crab meat were larger than those in the normal canned crab meat.

2) The amounts of free amino acids and sugars in the juice of browned canned crab meat were smaller than those in the normal canned crab meat.

3) The amount of arginine in free amino acids in browned canned crab meat was smaller than that in the normal canned crab meat.

4) The amounts of glucose and ribose in the juice of the browned canned crab meat were smaller than those in the normal canned crab meat.

5) Browned canned crab meat had a caramel odour and showed fluorescence under ultra-violet light.

Among these differences items 2)~5) agree with characteristics which are found in the "browning" of certain foods caused by the Maillard reaction. Accordingly there is strong basis for the deduction that the "browning" of canned crab meat is caused by the Maillard reaction.

2. *Effects of organic compounds on the "browning" of the canned crab meat*

If the "browning" of canned crab meat is caused by the Maillard reaction between amino acids and sugars, the aqueous solutions extracted from crab meat (which were free from protein or sugar) will be caused to become brown in color by heating after the addition of some kinds of amino acids or sugars which are commonly contained in crab meat. The author has studied the kinds of amino acids and sugars influencing the occurrence of "browning"[14].

The finding that basic amino acids accelerate the "browning" agrees with the observations of Willits[100] and Lents[101].

The present author has observed that the "browning" of the canned crab meat was prevented by the addition of sodium bisulfite which has been known as an inhibitor of the Maillard reaction. From the results obtained as above noted, the "browning" of canned crab meat can be considered to be caused by the Maillard reaction.

B. Factors influencing the "browning" of canned crab meat

1. *Relation between the "browning" of canned crab meat and the freshness of raw crab meat from which the canned crab is prepared*

According to Lea[33] and Hannan[34], in the "browning" of foods caused by the Maillard reaction, the larger the amounts of amino acids or sugars present in the food are, the more the "browning" is increased. In Jones' study[102], it was shown clearly that the amounts of free amino acids, glucose and ribose increased during the storage of fish meat even at a low temperature. Therefore, the decomposing of the raw crab meat is considered to have a close relation with the "browning" of the canned crab in connection with the length of the time that the raw material was left before being processed. In fact, according to the present author's results, when the canned crab was prepared from unfresh raw crab meat heated according to the "high temperature boiling method", the canned crab meat always became brown in color.

The present author has studied the change of the amounts of various chemical components in which the "browning" of canned crab meat seemed to bear some connection with the lapse of the time during which the raw crab meat was left at 0°~3°C (Article II). According to the results, the pH value of the raw crab meat just caught was 6.8 and the pH value decreased to 5.5~5.9 with the passage of time of leaving for 45~48 hours, but pH value increased gradually with the passage of time beyond 48 hours. The amounts of volatile basic nitrogen (V.B.-N.), amino nitrogen and reducing sugar increased with the lengthening of the time of leaving: especially were the variations remarkable in the body section (shoulder meat) which was kept in touch with viscera. The amounts of basic amino acids, glucose or ribose which are factors causing the "browning" of the canned crab meat increased remarkably when the raw crab meat without carapace was left. This is considered to be caused by the autolytic action or enzymatic action in the viscera. Oshima and Kondo[103] made clear that amylase, maltase, lipase and protease are to be found in the viscera of crab. According to their results the amylase is the most active on the acidic side such as at pH 5.9~6.2. Fujikawa[104] has observed that the amylase is still active at a low temperature, such as –13°C. The present author observed the variation of the amount of glycogen in the raw crab meat in relation with the time of leaving the crab meat. In raw fresh crab meat, about 0.25% of glycogen was present, but when the crab meat was left at 0°~3°C the glycogen was decomposed and its amount decreased to 0.176% after a lapse of 48 hours and to 0.14% after 92 hours; to the contrary, the amount of reducing sugar increased. Therefore, if the crab meat is left for a long time, its pH value decreased to reach the optimum pH values for activity of amylase or glycogenase, so that glycogen was decomposed to glucose. In the prolongation of the period of leaving the meat, nucleic acid in meat protein

will also be degraded by RN-ase[105] and other enzymes to its constituent mononucleosides, and the formation of free ribose from the mononucleosides will be accelerated by riboside hydrolase by the variation of the pH value of the meat which is left[31].

2. *Relation between the conditions of the preservation of raw crab meat and the "browning" of the finished canned product*

The degrees of the "browning" differ depending upon the condition of preservation of the raw crab carcasses, *e.g.* the degree of "browning" of canned crab meat which was prepared from carcasses preserved in ice water was not so great as that prepared from carcasses preserved in crushed ice (Article V). Notably, there was almost no "browning" of the canned crab meat prepared from raw crab carcasses preserved in ice water containing 0.05% sodium bisulfite, 0.3% sodium citrate or 0.3% sodium borate. The reason may be explained by the fact that the amount of chemical components flowing out of the material is affected by the pH value of the soaking solution. As stated in the previous paper (Article V) the degree of "browning" is generally influenced according to the amounts of the chemical components (amino acid, sugar and metals) flowing out from the crab meat. Certainly the amounts of those chemical components flowing out from the carcasses soaked in ice water are larger than the amounts from material left with crushed ice. Therefore the amounts of the chemical components remaining in crab meat without flowing out are smaller in case of the meat stored in ice water than in the other lots treated with crushed ice. Furthermore, the amounts of the chemical components flowing out from the crab meat are influenced remarkably by the pH value of the solution in which the meat is soaked.

The amounts flowing out were small on the acidic side, pH 5~6, and were large on the alkaline side. This is due to the fact that the isoelectric points of soluble protein in crab meat and haemocyanin in crab blood are in the range of pH 4.5~5.1[10,38]. When the crab meat is kept at those pH values, the amounts of total nitrogen, amino acid nitrogen and Cu which flow out from the crab meat are the smallest. In other words, in such a case the various chemical components remain abundantly in crab meat. On the other hand, amylase in crab meat is the most active at pH 5.9~6.2 and glucosidase is the most active in pH 5.0, so polysaccharides in the crab meat kept on the acidic side are decomposed quickly to glucose[103,106]. It was made clear in the "browning" reaction in the model system that the formation of N-glucosides by the condensation of glucose and arginine is accelerated with the descending of the pH value.

In fact, the canned crab prepared from raw crab meat soaked in acidic ice water (pH 5.0) by adding 0.3% citric acid or hydrochloric acid became always brown. On the contrary, "browning" of the canned crab meat prepared

from raw crab meat preserved in ice water adjusted to the alkaline side by the addition of 0.05% sodium bisulfite, 0.2% sodium citrate or 0.2% sodium borate did not occur (Article V).

3. *The relation between the "browning" and chemical components in crab blood*

In the studies of the "browning" reaction, Jones[107] has made clear that Cu ion acts as a catalyzer in carbonyl-amino reaction. According to his results, in the "browning" reaction of cod meat, if the meat is pH 6.8 and there is 5 *p.p.m.* of $CuSO_4$ in the meat, the "browning" was accelerated. Mohammed, Olcott and Fraenkel[108] and Tarr[28] have obtained the same results. Shewan[109] has also observed that the "browning" of salted cod meat was accelerated by Cu ion. In fact, the "browning" of canned crab meat with body fluid was more remarkable than that without the fluid. In this case, the amount of Cu in the juice of the former can was larger than that of the latter. In blood of raw crab, there exists haemocyanin which is a chromoprotein containing Cu, of which the amount is 0.16~0.19% (Article IX). According to the results obtained by the present author, beside Cu ion, Fe ion also acts as an accelerator to the "browning" of canned crab meat (Article II).

In conclusion, if the amount of Cu is above 2.5 *mg*% or Fe is above 5~10 *mg*% of the weight of the crab meat, the "browning" reaction of the canned crab meat is surely accelerated.

4. *Relation between the "browning" and the difference of peeled crab and hard shell crab*

It was observed that the degree of "browning" of canned crab meat which was prepared from hard shell crab was larger than that prepared from peeled crab (Article III). This may be due to the difference of chemical components taking part in the "browning" reaction of the two creatures.

In fact, the peeled crab flesh has a larger amount of water-content than the hard shell crab flesh, and the amounts of organic compounds of the former are smaller than those of the latter. Especially are the amounts of free amino acids and reducing sugars (glucose or ribose) in the peeled crab flesh smaller than in the hard shell crab flesh. From those differences in the compounds, it is considered that the "browning" of the canned crab meat prepared from the hard shell crab becomes larger than that prepared from the peeled crab.

5. *Relation between the "browning" of canned crab meat and the conditions of boiling crab carcass*

In the manufacture of canned crab, the boiling process is an important step in respect to the removal of the meat from the crust; also the qualities of the canned product depend on the results of the boiling process[110,111]. Thus, it is assumed that the "browning" is noticeably influenced by the conditions

of the boiling process. From the results described in Article VI, it was determined that the degree of "browning" of canned crab meat is remarkably influenced by the conditions of the boiling of the meat: that influence is especially dependent on the pH value of boiling water, on kinds of chemical compounds dissolved in the boiling water, and on the temperature of the boiling water.

The canned crab meat prepared from the material which was boiled by "high temperature boiling method" does not become brown, provided the raw meat is fresh. The amounts of chemical components which take part in the Maillard reaction, such as free amino acids and reducing sugars are smaller in fresh crab meat than the amounts in unfresh crab meat (Article III). When the pH value of the crab meat decreases, the ratios of its dehydration or shrinkage become large[48]. When such unfresh crab meat is boiled at 100°C, the surface skin of the meat in the crust will be heat-coagulated rapidly, so the flowing out of chemical components in the meat will be prevented. As the result those components will be accumulated within the meat and the amounts will increase, so the Maillard reaction will be caused rapidly by successive processing (heat sterilization).

The canned crab meat prepared from crab carcasses boiled by "low temperature boiling method" (below 70°C) easily browns, even if fresh raw carcasses are used. This may be explained as follows. The autolytic enzymes are not destroyed unless the meat is heated at 80°C for more than 10~15 minutes[51]. Thus, if crab carcasses are heated below 70°C, the activity of the autolytic enzymes remains in the meat. When the carcasses of raw crab are heated at 60°C for 10 minutes by "low temperature boiling method," the temperature of the center of the meat will reach a maximum of 55°~56°C. In view of the fact that the optimum temperature of the autolytic enzymes in crab meat is 45°~55°C[51], the temperature of the center of the meat under such heating conditions will be kept at the optimum for their activity in the crab meat (Article VII). Accordingly, even if fresh crab carcasses are used, the decomposition of the meat will be advanced during the boiling process at 60°C, also the action will continue in the boiled meat, and the chemical components connected with the Maillard reaction will increase. Thus the canned crab meat prepared from fresh raw carcasses heated by "low temperature boiling method" will become brown just the same as the canned meat prepared from unfresh raw carcasses heated by "high temperature boiling method." Furthermore, the "browning" reaction in this case may be accelerated remarkably by Cu ion; the amount of Cu in the juice of the browned canned crab prepared from the crab carcasses boiled by "low temperature boiling method" was found to be larger than that in the juice of the normal canned crab (not browned) heated by "high temperature boiling method." This is explained by the following fact; the amount of Cu which is dis-

sociated by heating from the haemocyanin in crab blood was large in the case of the heating of the crab blood at 50°~70°C (Article VIII and IX), also the amount increased accordingly with a prolongation of the period of leaving the raw meat (with falling of freshness). In fact, in the juice of the canned crab meat processed from the crab carcasses heated by "low temperature boiling method" (below 70°C), or from unfresh raw carcasses the amount of Cu was large, and the degree of the "browning" was also correspondingly large.

Thus the "browning" of the canned crab meat has an intimate relation with the methods employed in boiling the raw carcasses.

6. *Relation between the "browning" of the canned crab meat and pH values of boiling water and of the crab meat at the processing*

The degree of the "browning" of the canned crab meat is strongly influenced by the pH value of the boiling water and the kind of chemical compounds added into the boiling water.

Here, the author would like to emphasize the fact that the "browning" of canned crab meat is influenced remarkably by the pH values of the meat during the process of canning: *e.g.* by the pH value of boiling water in which the raw meat with crust is boiled in order to take out the meat from the crust by "high temperature boiling method" or "low temperature boiling method" and by the pH values of the boiled meat without crust which is filled into cans for the processing (sterilization in a retort).

For example, when the raw crab is boiled in acidic water by "high temperature boiling method" and the can filled with the boiled meat is processed at a high temperature (6 pounds pressure, 110°C), the canned content shows deep brown in color and its merchandise value is decreased by its poor elasticity. On the contrary, if the crab caracasses are boiled by the "high temperature boiling method" in the boiling water adjusted to the acidic region as described above, and the can filled with the boiled meat is processed at the same temperature in the acidic region of the meat, the canned content shows white in color. On the other hand, when the crab carcasses are first heated by the "low temperature boiling method" in the alkaline region and then the boiled meat taken out from the crust is heated for hardening in the acidic region, and the resulting meat is placed into cans and is processed, the canned content shows white in color. As a result of the use of other procedures (*e.g.* the boiling water used first at "low temperature boiling" was acidic and the water used at the hardening-boiling was acidic or alkaline), the canned content showed deep brown.

In order to make clear the relations between the "browning" of the canned crab meat and the pH values of boiling water or the meat at boiling procedure or the processing, the results described above are summarized in Table 45.

It is assumed that the results as to "browning" as described above are caused by peculiarities of the chemical properties of crab meat. The solubility, dehydration ratio and hydrating affinity of the meat are remarkably influenced

Table 45. Relation between the "browning" and the pH values of boiling water or the meat during boiling procedure or processing

(1) High temperature boiling method

Process — Boiling carcasses at 100°C →	Removing crust →	Color of boiled meat →	Processing the meat →	Color of the can content
Reaction of boiling water			Reaction of meat	
Acidic		White	Acidic	White
			Alkaline	Brown
Alkaline		White	Acidic	Brown
			Alkaline	Brown

(2) Low temperature boiling method.

Process—Boiling carcasses at 60°C →	Removing crust →	Hardening boiling at 100°C →	Color of boiled meat →	Processing the meat →	Color of the can content
Reaction of boiling water				Reaction of meat	
Acidic		Acidic	White	Acidic	Pretty brown
			White	Alkaline	Brown
Acidic		Alkaline	White	Acidic	Brown
			White	Alkaline	Brown
Alkaline		Alkaline	White	Acidic	Brown
			White	Alkaline	Brown
Alkaline		Acidic	White	Acidic	White
			White	Alkaline	Pretty brown

by either the pH value or temperature of the solution as described below. When the crab meat was soaked in a solution of pH 5.0, the amount of chemical components which flowed out from the meat showed minimum, while heat shrinkage of the meat and dehydration ratio became maximum[38,48]. Also, solubility, dehydration ratio and heat shrinkage of the crab meat were influenced by the heating tempreature[38,48]. Each amount of total-N, amino-N, reducing sugar and Cu compounds which flower out from the meat was known to prossess the maximum value in the boiling temperature range between 40°~60°C (Article VI)[38]. At a temperature of more than 60°C, the shrinkage of the crab meat progressed rapidly together with its dehydration[48]. It is considered

that the chemical components, such as amino-acids, sugars and Cu compounds of body fluid, which cause the "browning," flow out as an extract in large amount from the meat boiled on the alkaline side. On the contrary, if the meat is boiled on the acidic side, coagulation of the protein rapidly advances on the surface part of the meat. When the surface part of the meat was coagulated during the boiling, the chemical components which cause the "browning" may be accumulated within the boiled meat in a large amount, and the degree of the "browning" of the canned meat may increase. This may be true because: (1) the solution containing sugars and amino acids is concentrated as in the dehydration of meat, (2) the condensation reaction between sugars and nitrogenous compounds easily goes toward the formation of N-glycoside, and (3) the "browning" reaction progresses easily to completion[89,90]. When crab meat is boiled or sterilized at a high temperature of 100°C or above, the degree of "browning" of the meat is remarkably influenced by the pH value of the meat[17]. If that value of the meat in the can is in the alkaline region, the "browning" is remarkably increased, but the degree of "browning" of the meat on the acidic side is slight.

In such a cases, if the crab caracasses are boiled in water of which the pH value is adjusted to the alkaline region by the addition of sodium bisulfite, sodium borate or sodium citrate and the resulting meats are processed on the acidic side, the resulting products show pure white in color.

This fact is depending on two actions of reagents which are added to the boiling water; the flowing amounts of chemical compounds taking part in the "browning" become large when the carcasses are boiled in the boiling water containing those reagent as just noted, and the other is that those reagents act itself as an inhibitor for the "browning" reaction[14] (Articles V and VII). According to Wolfrom[112], Stadtman[80] and Hodge[113], sodium bisulfite combine with sugars and then the combining reaction between amino acid and sugar is interrupted, so the "browning" will not be generated. The reason for the preventing action of sodium borate upon "browning" of the canned crab meat will be explained by Nelson and Steinberg's result[114]. They reported that sodium borate prevents the oxidation of polyphenol in the "browning" of dried apple, and that the condensation of amino-sugar after the middle stage is not promoted.

7. *Relation between "browning" of canned crab meat and the decomposition of standing boiled crab meat*

When the crab meat after boiling is left for a long time and then packed into cans, the crab meat in the cans is apt to turn brown; this inclination is remarkable in the cans prepared from the boiled meat subjected to "low temperature boiling method." Therefore the present author has recommended that the boiled meat subjected to "low temperature boiling method" should be boiled again for hardening the meat as soon as possible. If the boiled meat

once subjected to "high temperature boiling method" is left more than 20 hours at 10°~15°C and is processed, the canned product becomes brown[15].

When the boiled meat subjected to "low temperature boiling method" is left, the meat is decomposed by autolytic enzymes which remain after boiling, in cooperation with bacterial action, to yield various chemical components taking part in "browning"[38,48]. On the other hand, when the boiled meat subjected to "high temperature boiling method" is left, it will be attacked only by bacteria. However, if the meat is left for a long time at a suitable temperature (room temperature) for bacterial activity, the boiled meat is decomposed to yield various chemical components which concern "browning."

C. Demonstration of "browning" of canned crab meat resultant from the Maillard reaction

In the course of this study, the author has suggested that the "browning" of canned crab meat is caused by the Maillard reaction and he has studied the factors influencing the "browning" phenomena in the canned crab meat.

For the purpose of checking the suggestion, the present author will demonstrate that "browning" is caused by the Maillard reaction between amino acids and sugars by testing the intermediate products in various stages of the course of the reaction. The author isolated the typical products formed in each stage of the course of "browning" of canned crab meat and found that the isolated products agreed in their properties with the typical products formed in each stage of the "browning" reaction of the model system.

1. *Isolation and identification of N-glucosides*

From browned boiled or canned crab meat, white hygroscopic powder was isolated. It was ascertained that the white powder showed a weak reducing power, was positive to the ninhydrin test, and became brown on heating. Those properties agreed with Hodge's findings[59]. The total amounts of nitrogen in the white powder isolated from browned boiled crab meat and browned canned meat were 17.09% and 18.77%, respectively. Those amounts almost agreed with the total amounts of nitrogen in the condensates prepared from arginine and glucose by Hamamura[64], or from arginine and ribose by the present author (Article X). The total amount of nitrogen in the white powders obtained as above, differs slightly from the total amounts of those in the condensates from histidine and glucose or histidine and ribose. Paper chromatographic detection was made from the white powders isolated from boiled or browned canned crab meat, and in those hydrolyzates, arginine as amino acids, and glucose and ribose as sugars were found to be chemical components. The Rf values of the white powders (0.075~0.09) agreed with corresponding values of the condensates synthesized from those components (amino acids and sugars as just noted).

From the consideration based on the nitrogen contents or the chemical components existing in both white powders separated from the boiled crab

meat or browned canned crab meat and the synthesized N-glycosides, those white powders may consist of the condensates of arginine and glucose or ribose. The formation of N-glycoside is affected by the pH values of the mixture of amino acid and sugar and the condition of heating the mixture; the formation is accelerated by heating (at 100°C) or by reducing of pH value to acidic side in the mixture. Therefore free amino acids and sugars in crab meat are considered to combine with each other to form N-glycoside in the initial stage of the "browning" of canned crab meat.

2. *Isolation and identification of fluorescent substance*

The author has observed that the canned crab meat which was prepared from boiled crab meat showing fluorescence under ultra-violet light became brown in color (Article XI). Also, when the condensation products between amino acids and reducing sugars, N-glycosides, were heated, fluorescence generated in the heated matter which had not yet become brown in color. Thereupon the fluorescent substance isolated from the boiled crab meat was compared, by the estimation of absorption peaks with that formed in the condensation of amino acids and reducing sugars system (Article XI). The absorption peak appeared at 283 $m\mu$ in the fluorescent substance prepared from browned boiled crab meat; on the other hand, the absorption peak appeared at 264 and 280 $m\mu$ in that obtained by heating the condensate of arginine and glucose, and at 283 $m\mu$ in that of arginine and ribose. Thus the absorption peaks are almost the same. The fluorescent substance obtained from the browned boiled crab meat is considered to be the same as the substance formed by the heating of the condensate of amino acids and reducing sugars. The production of fluorescent substance in the boiled crab meat indicates the progress of the Maillard reaction.

3. *Identification of hydroxymethylfurfural in the browned boiled crab meat*

In addition to the fluorescent substance, hydroxymethylfurfural (H.M.F.) or reducton is known to be produced in the middle stage of the Maillard reaction[60,83].

Here, the author made tests for the presence of H.M.F. in the browned canned crab meat by means of paper chromatography, polarography and observation of the absorption spectrum. Furthermore, the formation of H.M.F. was detected in the "browning" in the model system (the heated condensate product of arginine and glucose or ribose) by the same method as employed for the detection of H.M.F. in the browned canned crab. The results obtained in both cases were almost the same (Article XII). Also, those results obtained in the detection of H.M.F. agreed with the results obtained by Gottschalk and Partridge[60], Wolfrom and Schuetz[87], Täufel and Iwansky[81] and Nomura[83]. Thus, the formation of H.M.F. has been proven in the course of the "browning" reaction of the canned crab meat (Article XII). On the

basis of those results such "browning" is considered to be caused by the Maillard reaction.

4. *Isolation and identification of melanoidins*

Melanoidins are not simple compounds; they are nitrogenous brown pigment substances which are obtained by combination between amino acids and reducing sugars upon heating[22,90]. The author has isolated the brown pigment substances from browned canned crab meat, of which substances the properties were compared with those of the brown substances obtained from the heated condensates of amino acids and reducing sugars in respect to the general properties, absorption spectrum and paper chromatography (Article XIII). No difference was found in the properties between melanoidins separated from the browned canned crab meat and those obtained from the condensation of amino acid and sugar by heating. Furthermore, the general properties of melanoidins obtained by the present author (Article XIII) agreed with the results reported by Enders[93]. In the result, the melanoidins which were final products of the Maillard reaction were identified in the "browning" reaction of the canned crab meat; the "browning" of the canned crab meat was thus ascertained to be caused by the Maillard reaction.

Accordingly, in the comparison of the respective products formed in each stage in the course of the Maillard reaction in the model system with those products obtained in the "browning" of canned crab meat, the "browning" of canned crab meat is considered to be caused by the Maillard reaction by the combination between amino acids and sugars in the processing of canned crab.

D. Mechanism of the "browning" reaction in the canned crab meat.

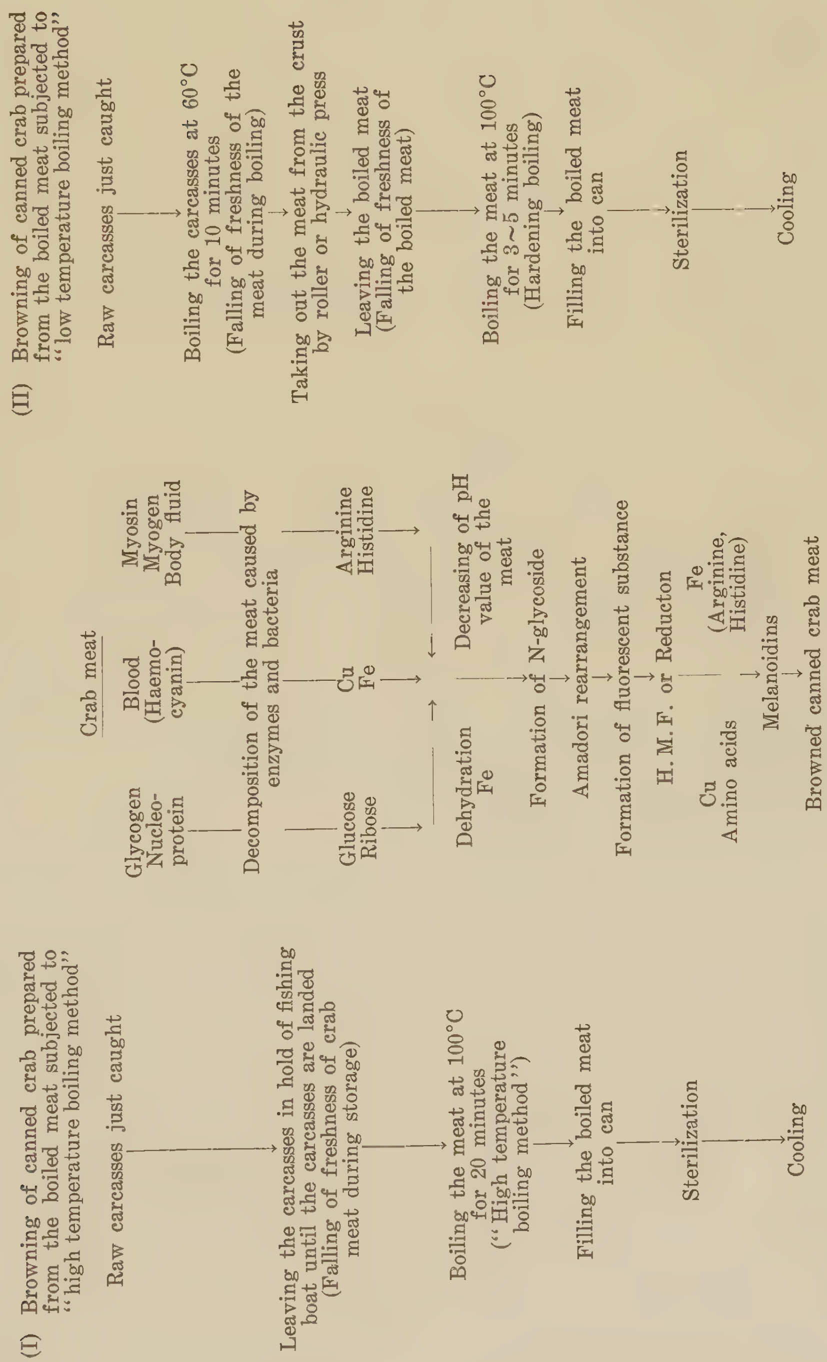
As a cause of the "browning" reaction in the canned crab meat the Maillard reaction between amino acids and sugars has been proven. The author has explained the mechanism as shown in Scheme 4.

As seen in Scheme 4, the proceeding of "browning" of canned crab meat is divided mainly into two sorts. One of them is the "browning" in canned crab meat prepared from unfresh raw material boiled by the "high temperature boiling method." The other course is that occurred in canned crab meat which was processed from fresh raw material boiled according to the "low temperature boiling method" (Article VIII).

1. *"Browning" of canned crab meat prepared from unfresh raw material subjected to treatment by the "high temperature boiling method"*

When unfresh raw crab which was left for more than 48 hours at about 3°C after being caught was used for the processing, the canned content always became brown. During storage of the raw crab meat, it was decomposed by autolytic enzymes and bacteria; the pH values of the meat decreased to 5.5 which is optimum for enzymatic activity, the amount of glycogen in the crab meat decreased in accordance with the increasing of glucose, the amount of ribose which is separated from nucleo-protein in crab meat increased, and

Scheme 4. Mechanism of "browning" reaction in the canned crab meat



amino acids (such as arginine and histidine) were separated in a free state from the proteins of the crab meat. If the pH value of the meat decreases to the acidic side, meat protein and haemocyanin in blood will coagulate and their solubilities will decrease, therefore the flowing amount of body fluid (blood) will decrease. Thus, large amounts of amino acids and sugars and Cu (in haemocyanin) which are concerned with the "browning" of canned crab meat will be accumulated in crab meat. When the crab meat containing a large amount of those chemical compounds is heated by the "high temperature boiling method" (100°C, 20 minutes), the formation of N-glycoside by combination of amino acids and sugars will be accelerated by the dehydration of the meat. This statement is supported by the fact that N-glycoside is separated from the boiled crab meat (Article X).

When unfresh crab meat is boiled by the "high temperature boiling method," the boiled crab meat shows fluorescence under ultra-violet light. The change from N-glycoside to a fluorescent substance is considered to be caused by rearrangement of N-glycoside[59]. After the boiled crab meat has been taken from the crust, and when the meat having fluorescent substance is placed in a can and then processed, the fluorescent substance will be changed to H.M.F. (or reducton) by dehydration of the meat as a result of the heating. Hydroxymethylfurfural will change to melanoidins as a result of the combination of arginine and histidine isolated from the crab meat in the can during the processing. In this case, the presence of Fe in the meat, or of Cu in the blood particularly accelerates the change, as described in Article II. Gottschalk and Partridge[60] have also showed the formation of H.M.F. from amino acids and sugars by paper chromatography.

2. *"Browning" of canned crab meat prepared from fresh raw material subjected to the "low temperature boiling method"*

Even if fresh crab is used on the floating cannery, when the canned crab is produced from the boiled meat heated according to the "low temperature boiling method" (60°C, 10 minutes), the canned content will become brown. This phenomenon may be explained by results obtained as described in the previous Article VIII as follows: when fresh crab carcasses without carapaces are subjected to the "low temperature boiling method," the center of the crab meat in the crust will be maintained during boiling at 45°~55°C which is the optimum temperature for activity of the autolytic enzyme. As the autolytic enzymes are not destroyed by this boiling, the boiled meat is decomposed by enzymes and bacteria until the meat is treated by the hardening-boiling process method; during this processing the boiled meat is taken out from the crust and the meat without crust is washed. Thus, even if fresh raw meat is used for canning, a large amount of amino acids and sugars is accumulated in the half-boiled meat. Then N-glycoside will become melanoidins *via* intermediate products such as fluorescent substance and H.M.F. as described above. The

presence of Cu in blood also accelerates the formation of melanoidins. The Cu is dissociated more easily from haemocyanin in the crab blood (in meat) subjected to the "low temperature boiling method" than in that subjected to the "high temperature boiling method" (Article IX). In order to prevent the dissociation of Cu, the crab carcass must be heated above 80°C.

E. Methods of prevention of the "browning" of canned crab meat

From the consideration on the mechanism of "browning" of canned crab meat, the prevention methods are divided into three sorts as follows:

(a) Prevention method based on the improvement of preserving the carcasses in a fresh state without decomposition until the carcasses are landed.

(b) Method of prevention by improvement of the conditions of boiling the crab meat (fresh or unfresh crab carcasses).

(c) Prevention method based on the selecting and picking out the boiled meat having fluorescence before filling into cans.

1. *Method of prevention based on the improvement of preserving carcasses in a fresh state without decomposition until the carcasses are landed.*

When unfresh carcasses were used, the canned crab meat (part of white meat) prepared from meat boiled by the "high temperature boiling method" became brown. Then what degree of freshness of the raw meat is the limit for the preparation of canned crab without "browning"?

The limit is considered to be 15~20 *mg%* in the amount of volatile basic nitrogen in the raw meat, from the results obtained by Tanikawa and the present author[115]. The time needed for the falling of the freshness of the meat to the limit is remarkably affected by the temperatures of storing the meat, because both the action of bacteria and of autolytic enzymes is affected by temperature. The conditions of storing the meat (temperature and time) in which the freshness of the raw meat falls to the limit were observed to be within 48 hours at 3°C, 36 hours at 7.5°C, and 9 hours at 20°C[15,116]. If the canned crab was prepared from the raw carcasses which had been stored outside of the limited conditions of storing as noted above, the resulting canned products always became brown in color. When the fresh raw carcasses which were stored within the limited condition cannot be used directly for the manufacturing of canned crab, by what method can the raw carcasses just caught on ship be preserved until the materials can be landed at a shore canney?

The present author has compared the qualities of the canned crab meat as affected by two storing methods; one is the storing of the meat with crushed ice and the other is storing the meat in ice water (Article V). The latter was observed to be better than the former method. This is considered to depend upon the facts that the carcasses stored in ice water in the hold were uniformly cooled and kept at a low temperature without being subjected

to atomospheric temperature, also blood or chemical components concerned with "browning" were easily removed from the carcasses in the ice water.

In the case of storing raw carcasses in ice water, if the pH value of the water was adjusted, or if some chemical salts were added, the prevention of "browning" was observed to be more effective than in the case of being storing in ice water alone. It was observed that when crab carcasses were stored in ice water adjusted at pH 8.5 by the addition of NaOH alone or with other chemical salts, for example, sodium bisulfite in the proportion of 0.05%, sodium citrate in 0.2%, or sodium borate in 0.2% or sodium hypochlorite in 0.01%, "browning" of the canned crab meat was prevented (Article V). Here, the degree of the "browning" of canned crab meat prepared from the raw carcasses which were stored under various conditions were compared.

The canned contents prepared from raw crab carcasses stored and soaked in alkaline ice water or ice water containing chemicals *viz.* sodium bisulfite, sodium citrate, sodium borate and sodium hypochlorite were whiter than those stored in only ice water or acidic ice water. Among the chemicals, sodium hypochlorite was the most effective (Article V). The reason why the "browning" of the canned content was prevented by storing the raw carcasses in an alkaline ice water, depends on the organic acids being generated during the storage of the raw crab meat were neutralized by adding alkali; the action of the autolytic enzymes of which the optimum pH is on the acidic side was brought about slowly by adjusting the meat to the alkaline side, and also, chemical components concerned in the 'browning" reaction easily flowed out.

The reason why the addition of sodium bisulfite to ice water can prevent "browning" of the canned content is considered to be that sodium bisulfite can prevent bacterial growth and can bleach the brown pigment which generates in the crab meat during the processing[117]. According to Tillitson[118] and Demolis[119], bisulfite reacts with the carbonyl group of sugars and makes them inactive; so bisulfite is an inhibitor of the Maillard reaction. The reason why storing the raw carcasses in ice water containing sodium citrate prevents "browning", depends on sodium citrate inhibits the coagulation of blood in crab meat, so the blood can easily flow out from the meat, and the influencing Cu in blood is removed (Article V). The reason why "browning" is prevented by use of ice water containing sodium borate, depends on the sodium borate reacting with the carbonyl group of sugars to make a complex-salt interrupts the combination with amino acids[120]. Nelson[114] has observed that the use of borax has been effective in preventing the "browning" of dried apples. It is desirable that chemicals for preventing the "browning" of the canned crab meat possess certain properties; such as that the antiseptic action be powerful, that the pH value of the chemicals be alkaline in solution and that the autolytic action be interrupted by chemicals which easily permeate the meat.

Next the author will explain why sodium hypochlorite was the most effective

for the prevention of the "browning". The reason is that sodium hypochlorite has antiseptic action and prevents occurrence of autolysis[121]. Its antiseptic action is especially largest at pH 8~9 and the action is kept for a long time under the maintenance of these pH values.

2. *Method of prevention based on the improvement of the conditions of boiling the crab meat (fresh or unfresh crab meat)*

As described above in Article VIII, if canned crab is produced from unfresh raw carcasses through the usual processing (boiling by the "high temperature boiling method"), the products always become brown in color. When unfresh raw carcasses have to be used for the manufacturing of canned crab, it is important in prevention of the "browning" to remove the chemical components which take part in the 'browning" by some method. For example, raw crab meat is treated as follows: the raw carcasses are treated in alkaline water by the "low temperature boiling method," then the half-boiled meat is boiled in an acidic solution for the purpose of hardening the meat, and finally the meat in the can is processed on the acidic side (See above section B, (6) in this Article). By boiling crab carcasses in alkaline water at a low temperature (60°~80°C), the free amino acids, sugars and body fluid (blood) will flow out from the meat. By the last processing of the boiled meat on the acidic side, the progress of the "browning" reaction after the middle stage will be inhibited. So the canned crab meat will be white.

In the boiling process of the "low temperature boiling method," if some complex salt-making reagent (*e.g.* a polyphosphate, *Calgon, Curafos* is added to the boiling water, the degree of the "browning" of canned product is decreased and also the formation of crystals of $MgNH_4PO_4 \cdot 6H_2O$ (so called "struvite") is prevented[116]. This effect may be considered to be caused by the fact that water soluble components in the meat, such as amino acids, sugars and especially Cu, easily flow out from the meat during the boiling. On the other hand, when the canned crab (boneless can) is prepared from fresh crab carcasses by the "low temperature boiling method" (in this case, chemical reagents are not used in the boiling water), the canned content becomes brown in color. If this defect of "browning" is corrected, the boiling method will be satisfactorily determined. If the carcasses are boiled at 80°C for 10 minutes, the enzymatic action is stopped and the dissociation of Cu in blood is remarkably reduced. But it becomes difficult to take out the meat from the crust without deformation of the meat, because the meat becomes too hard. The present author has observed that in the case of crab carcasses boiled at 80°C for 6~7 minutes, or 75°C for 7~10 minutes, the meat may be removed without deformation of the meat from the crust (Article VIII).

Here, the author proposes a new improved "low temperature boiling method." The new method consists of the first "low temperature boiling" in slightly alkaline water (pH 8.0~8.5, 80°C, 6~7 minutes), taking out the meat by

roller or a hydraulic press, the second "low temperature boiling" (80°C, 5~7 minutes) in also slightly alkaline water, cooling and washing the boiled meat in water or in water containg 10 *p.p.m.* sodium hypochlorite, and hardening boiling in acidic water (100°C for 1~2 minutes). The remaining part of the processing is the same as the usual method. According to this new method, the first boiling is done with the purpose of removing the chemical components concerned with 'browning", whilst the second boiling is done to destroy the action of autolytic enzymes and to came the flowing out of the chemical components as described repeatedly above. The "hardening-boiling" is for the same purpose as described above.

The author has tried to manufacture canned crab from crab carcasses having various degrees of freshness according to this method. After the opening of canned products prepared by the new method, the "browning" and other qualities were inspected by factory technicians.

In the comparing the canned crab prepared by new method with control cans prepared by the usual method ("high temperature boiling method") from the same degree of freshness of crab carcasses, the former cans prepared from fresh or unfresh carcasses had not browned meat or juice, *i.e.* the meat was white and the juice was transparent, but the latter cans prepared from unfresh carcasses became brown.

In the above test, the temperature and duration of the first boiling were considered to be good at 80°C for 5 minutes. If the size of crab carcasses is large, the boiling time must be increased to 7 minutes. The "browning" is not influenced by the kinds of reagents added to the first boiling water, but in the second boiling the effect appeared. In this case, employment of the combination of polyphosphate (complex salt-making reagent) with the other chemical compounds is more effective for the prevention of "browning" than that of each compound separately. In this case, the combination of polyphosphate and sodium borate is more effective than that of polyphosphate and sodium citrate. This is considered dependent on the facts that borate combines with sugar making a borate-complex, whilst polyphosphate combines with Cu in blood to make a Cu-complex, and that those complexes are removed by the next washing. On the other hand, citrate acts only to make the boiling water alkaline and to dissolve Cu ions out easily. Therefore, use of the combination of borate and polyphosphate in boiling water is the best, but the use of borate in foods is not permitted, so the use of citrate and polyphosphate together is next desirable.

As short a time as possible for the "hardening-boiling" is good, 1~2 minutes, to harden the meat. The longer the hardening boiling time is, the larger the ratio of dehydration becomes, and the harder the meat becomes. The pH value of the hardening-boiling water is best to be acidic (pH 5.5~6.0) in order to prevent the development of the "browning" reaction. But if the pH value is

below 5.0, the ratio of dehydration of boiled meat becomes larger, and causes the acceleration of the formation of N-glycosides, whereupon "browning" becomes greater. The use of borate is not permitted by the Drug and Food Administration. Sodium citrate should be used in spite of its smaller effect.

3. *Prevention method based on the selecting and removal of the boiled meat having fluorescence before filling the cans*

It was observed that if boiled crab meat containing a fluorescent substance under ultra-violet light is put into cans and processed, the canned meat always becomes brown (Articles IX, X). In fact, if the fluorescent substance is extracted with alcohol, and the extract is heated under the same conditions as those in the processing of cans, the extract became brown and the degree of the "browning" is proportional to the degree of fluorescence (Article X).

Here it was found that if some part of boiled crab meat having a fluorescent substance is picked out, the canned crab meat will be white, and the occurrence of browned canned crab meat will decrease. This is passive, but it is considered to be one prevention method.

SUMMARY

The color of normal commercial canned crab meat is generally white, but in the canned crab prepared by the usual processing ("high temperature boiling method") in land canneries in Hokkaido, or in that prepared by "low temperature boiling method" on floating canneries, it is occasionally observed that the white meat has turned partly to brown in color. Because of this so-called "browning" phenomenon in canned crab meat, the market value of canned crab may be so reduced. This phenomenon has raised a serious question in the canned crab industry. The author has studied this "browning" phenomenon and come to the following conclusions.

(1) Canned crab meat did not become brown when fresh raw crab carcasses had been used and subjected to the usual boiling method ("high temperature boiling method"), then processed.

(2) Canned crab meat always turned to brown when unfresh raw carcasses had been used and subjected to "high temperature boiling method," and then processed.

(3) When "low temperature boiling method" was employed, the canned products became brown, which were processed from not only unfresh, but also from fresh carcasses.

(4) "Browning" was considered to be caused by the combination of amino acids and reducing sugars in crab meat during the processing (Maillard reaction) from the following experimental results:

1) If either one of both amino acid and reducing sugar was removed from the raw crab meat as result with acetylation or fermentation, the meat of the finished product did not turn to brown.

2) From boiled crab meat or browned canned crab meat measures were taken to separate, N-glycosides which were formed in the initial stage of the Maillard reaction.

3) Fluorescent substance and hydroxymethylfurfural (H.M.F.) which are intermediate products of the Maillard reaction were separated from boiled crab meat or browned canned crab meat.

4) Melanoidins which are the end products of the Maillard reaction were separated from browned canned crab meat and their absorption peak appeared at 280~284 $m\mu$. Other properties were also compared with those of melanoidins obtained from the model system, and both groups of properties were almost identical with each other.

(5) The amounts of amino nitrogen and reducing sugar in browned canned crab meat were generally larger than those in normal canned crab meat. On the contrary, the amounts of amino nitrogen, reducing sugar and Cu in the juice of browned canned products were less than those in normal cans.

(6) "Browning" was more often observed in the canned product prepared from hard shell crab than in that prepared from peeled crab.

(7) Browned canned crab meat showed fluorescence under ultra-violet light. The degree of "browning" of the browned canned crab meat was proportional to the degree of the fluorescence of the meat.

(8) When boiled crab meat having fluorescent substance was processed into canned products, the meat always became brown.

(9) The "browning" phenomenon was influenced by the following factors:

1) Conditions of preservation of the raw crab carcasses: the preservation of raw carcasses was better in ice water than that in crushed ice.

2) pH of the ice water: the preservation of raw carcasses in ice water of which pH value was 8.0~8.5 was better than that in acidic water.

3) The presence of metals: the presence of Cu in crab blood (body fluid) and Fe in crab meat accelerated "browning" of canned crab meat.

4) Remaining blood in the meat (due to insufficient flowing out): the larger the remaining blood in the meat was, the greater the degree of "browning" became.

5) Boiling methods and conditions of boiling the crab meat (temperature, time, and pH of the boiling water).

6) The longer the time to leave the meat after boiling, especially when subjected to the "low temperature boiling method," the larger the degree of "browning" became.

7) Processing temperature and time: the higher the processing temperature was, or the longer the processing time was, the more the "browning" of the canned crab meat was accelerated.

8) pH of boiled meat put into cans during the processing: if the boiled crab meat put into cans was acidic and processed, the canned crab meat became

white. On the contrary, if the boiled meat was processed on the alkaline side, the canned product became brown.

(10) From the factors influencing "browning," its mechanism is considered in two ways. One is the "browning" which occurs in canned crab meat processed from unfresh raw material subjected to the "high temperature boiling method" (in land canneries). The other is that which occurs in canned crab meat processed from fresh raw material subjected to the "low temperature boiling method" (on floating canneries).

(11) To prevent the "browning," three methods were proposed.

1) Prevention by improving the preservation methods of the crab carcasses so as to keep them in a fresh state until they are landed and processed.

2) Prevention by improvement in the boiling methods.

3) Prevention by picking out and discarding the boiled meat having a fluorescent substance, which is a precursor of the brown pigment substance, before putting boiled crab meat into cans.

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